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Symposium 1: Antimicrobials, Gene and Viral Therapies

O1-S

Harnessing biodiversity of Cas9 to expand genome editing toolbox

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The Cas9 protein guide RNA of Type II CRISPR-Cas defense systems of bacteria have been adopted as a robust and facile genome editing tool. They have been reprogrammed to cleave, nick or bind desired chromosomal DNA targets and used in a multitude of applications to edit genomic DNA and to modulate gene expression. Typically one system that derived from *Streptococcus pyogenes* (Spy) has been utilized. Since the guide RNA of Spy Cas9 may be easily reprogrammed to hybridize with many DNA sequences by altering the ribonucleotide content of the spacer, the PAM sequence becomes a constraint which restricts the sequence space available for genome editing applications. This becomes particularly evident in human therapeutic applications where, for example, an individual gene from a highly related gene family is to be modified. Given the diversity presented by Type II CRISPR-Cas systems, we posited that Cas9 orthologs may provide a rich source of biochemical and biophysical diversity that may be beneficial for genome editing through novel PAM recognition, improvements in specificity, or differences in DNA target cleavage pattern.

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O1-1

Functional mineral scaffolds are actively produced by dedicated biofilm cells to provide resistance to antibiotics

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So far, microbial biofilms were considered to be held together primarily by organic polymers, such as proteins, polysaccharides and nucleic acids. We recently reported that structured calcite minerals deposits play an active role in the development of biofilms, and contribute to the overall fitness of the community. Furthermore, our results indicated that these mineral scaffolds can act as diffusion barriers, and increase the resistance of the community members to environmental stress.

To better understand the molecular events involved in biomineralization, we now analyzed the transcriptome of the biofilm cells grown either in the presence or absence of a soluble calcium source promoting biomineralization. We exposed a role in biomineralization for membrane nucleation, several novel signaling cascades and an uncharacterized channel involved in the uptake of calcium. Furthermore, these results were consistent with our scanning transmitting electron microscopy analysis that revealed the storage of calcium carbonate occurs in a controlled environment within the bacterial cells.

Calcite minerals may serve as an efficient load-bearing foundation in complex structures, promoting the stability of the overall structure, while preventing the diffusion of small molecule solutes and antibiotics into the colony. We now study the roles of the candidate genes rising from the transcriptome in biomineralization and ask whether the compartmentalization of these gene products within the cells has an active role in mineral production. Our results shed light on an intriguing developmental process involved in biofilm formation. Furthermore, they offer novel targets to combat phenotypic antibiotic resistance of bacterial biofilms.

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O1-2

Development of co-cultivation concepts for beta cells and mesenchymal stromal cells to produce functional beta cell spheroids

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The standard therapy for diabetes is strict monitoring of the blood glucose level and the application of insulin. This therapy form leads to long term damages e.g. circulatory disturbances, and is a major burden for the patient. The failure of the insulin-producing

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beta cells in diabetics can be remedied by the replacement with whole pancreatic islets from donors or pseudoislets created by beta cells or pancreatic-differentiated induced pluripotent stem cells (iPSCs). This therapeutic approach deals with the hurdles that the transplanted cells have a short lifespan at the application site and that beta cells cultured as traditional monolayers lose functionality. Beta cell cultivation in a 3D format as spheroids/agglomerates, similar to the beta cell origin in the islets of Langerhans, preserves functionality. For a more complex and supporting 3D environment, beta cells can be co-cultivated with human mesenchymal stromal cells (hMSCs). hMSCs enhance the pseudoislet survival by strengthen the beta cells. The positive effect of hMSCs is facilitated by cell-cell interactions and the secretion of trophic factors. We evaluated different co-cultivation set ups of beta cells and hMSCs. The mutual cell contact was either given by direct cell-cell interactions, transmitted through the culture medium or a combination of both. Due to the high amounts of high-quality cells needed for cell therapy (106–1010 cells per dose), we aim the development of 3D bioreactor process, which provides necessary features for co-cultivation and fulfills the requirements of good manufacturing practice (GMP) and process analytical technology (PAT).

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O1-3

Investigation of regulatory mechanism of antimycin-type depsipeptide biosynthesis in *Streptomyces albus* S4

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Antimycins are potent inhibitors of mitochondrial cytochrome c reductase and were recently shown to inhibit the Bcl-2/Bcl-xL-related anti-apoptotic proteins that confer resistance of tumour cells to apoptosis-inducing agents.

The antimycin biosynthetic gene cluster was first described in *Streptomyces albus* S4. It contains the only one cluster-situated regulator, an ECF RNA polymerase σ factor AntA. It is unique because it does not appear to be controlled by an anti-σ factor. This led to speculation about mechanism(s) as to how AntA activity is modulated. Analysis of the AntA amino acid sequence revealed the presence of a di-alanine motif at its C-terminus that is a recognition sequence for the ClpPX protease and suggests ClpXP modulates the activity of AntA.

We provide evidence in support of this hypothesis and also demonstrate possible functional overlap between ClpX and another Clp-regulatory subunit, ClpA. We introduced a FLAG-tagged copy of the wild-type antA gene or a variant in which the C-terminal AlaAla motif was mutated to AspAsp (blocks Clp-proteolysis) into a *S. albus* S4 ΔclpXP mutant. Western blot analysis of protein extracts of the resulting strains demonstrated that stability of the AntA-AspAsp variant was significantly elevated compared to AntA-AlaAla. Next, we overexpressed the clpXP and clpAP genes in the same genetic background. Analysis of protein extracts demonstrated premature proteolysis of AntA in these strains, however, proteolysis of AntA in the strain with overexpressed clpAP was temporally delayed. Future work aims to address the in vitro stability of AntA in the presence of ClpXP.

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In vitro antioxidant and antimicrobial activities of extracts from medicinal plants

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The occurrence of mycoses generally associated with oxida-tive stress, constitute an important public health problem today. This outbreak is due not only to the increase of risk factors, tox-icity but also to the resistance of pathogens to the therapeutic molecules available. In the aim to explore the potential spring of new therapeutic molecules, efficient and with a wide spectrum of action, we have evaluated the antifungal and antioxidant, activity of extract from Cameroonian plants. Five Cameroonian medicinal plants were selected from 2 plants families (Annonaceae, and Lami-aceae) and investigated for bioactivities properties. From these plants, 12 crude extracts, 28 fractions, and 3 purified compounds were obtained by maceration and fractionation using organic sol-vents and their biological activities were evaluated. The antifungal activity was evaluated using the microdilution method; the anti-radical activity was evaluated following the scavenging potential of extract and fractions against the DPPH free radical, and their oxygen radical absorbance capacity. The chemical compositions analysis were determined using phytochemical screening for the extracts and total phenolics contents and total flavonoids contents for the fractions respectively. All the extracts except Monodora ternuifolia seeds inhibited the growth of yeasts with Minimum Inhibitory Concentrations (MIC) ranging from 1.87 to 15 mg/ml and exerted the strongest radical scavenging potential. The results achieved from this work demonstrate the presence of active principles against pathogenic yeasts, free radicals in some plant species investigated in this study. However, further investigations are required to ascer-tain these findings and formilize their eventual use to control the targeted affections

O1-5

Antibacterial activity of zinc oxide and copper oxide nanoparticles against Gram-positive and Gram-negative bacterial strains ss

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The aim of this work is to develop a thin layer of nanomaterials-based on metal oxide nanoparticles (CuO, ZnO) in order to measure and optimize their microcide activity for surface decontamination for possible specific applications at industrial scale. The analysis of several metal oxides on both elaboration and biocidal activity are carried out in terms of effectiveness, potential to be used in various supports (paints, food packaging, coatings, etc.) and indus-trial application. In our work we develop, as antibacterial agents, nanoparticles of zinc and copper oxide.

The synthesis of zinc and copper oxide nanoparticles is performed by the sol-gel method. This method has an advantage of using soft chemistry and synthesizing very pure nanomaterials, we obtained nanoparticles around 5 nm of diameter. The antibacterial studies were performed on Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using the disk diffusion method, which is based on the migratory effect of nanoparticle solutions inside a petri dish in a solid nutrient medium. It is a qualitative method that makes possible to determine the sensitivity of microorganisms to an antimicrobial substances. After 24 h of incubation, ZnO nanoparticles showed antibacterial activity with inhibition zones of the order of 33 mm, 33 mm and 32 mm and CuO nanoparticles showed inhibition zones of 44 mm, 36 mm and 39 mm respec-tively for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This approach is used to select the best compromise

between antimicrobial effectiveness and nanoparticles concentration for coating applications.

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O1-6

Infant with osteosclerotic metaphyseal dysplasia and TBX3 gene mutation

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A female neonate born of 3rd pregnancy, presented with poor cry at birth, inability to move limbs, feeding problems and respira-tory difficulty. Antenatally there was oligohydramnios and features suggestive of fetal hydrops. She was admitted and put on IV flu-ids, antibiotics and oxygen support. She had bowing in the limbs and X-rays showed metaphyseal bands. A possibility of skeletal dysplasia was kept, and Vit C, Calcium, and Vit D3 levels were done along with tests for infection. The Vitamin C levels were low and supplementation was given. The baby continued on ventila-tor for 3 months, with multiple problems like seizures, neonatal jaundice, recurrent lung collapse, gastroesophageal reflux, and had slow improvement. Later she was found to have subglottic stenosis. The mother earlier had a similar pregnancy with oligohydram-nios and intrauterine death at 34 weeks gestation. Second baby was alive and well. Keeping a possibility of Inherited skeletal dysplasia, especially osteosclerotic metaphyseal dysplasia(OMD), genetic testing by next generation sequencing was performed, cov-ering over 320 genes causing skeletal dysplasia. The child expired around 6 months age. A heterozygous variant was identified in exon 8 of TBX3 gene, which also causes ulnar mammary syn-drome. Since TBX3 is involved in Vit C metabolism and TBX3 along with TBX5 are involved in cardiac development, the variant identified here possibly points to TBX3 being the likely candidate gene for OMD.

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Symposium 2: Biofortification of Crops

O2-S

Exploiting plant long-distance signalling mechanisms in agriculture

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Plant roots sense drying soil and send long-distance signals (such as plant hormones) to the shoot to limit water loss (by closing the stomata and restricting leaf growth) and increase crop water use efficiency (yield produced per unit of water applied or transpired). These signals can be modified by altering irrigation volume (how much water), frequency (when water is applied), timing (the stage of crop development that water is applied) and placement (where on the soil the water is placed), allowing a potentially infinite number of possibilities to the practitioner (farmer). Irrigation science seeks to understand how these changes affect crop growth and quality, with increasing emphasis on ensuring these methods are applied on-farm. Thus irrigation scientists need to interact both with farmers but also water resources managers to secure agricultural production in a changing climate.

The agronomic impacts of specific irrigation techniques (such as partial rootzone drying and alternate wetting and drying) will be evaluated, in seeking to understand how basic science on long-distance signalling can be applied on-farm. Moreover, alternate wetting and drying of rice offers the potential of increasing crop nutrient use efficiency and limiting the emission of potent greenhouse gases. Relatively simple irrigation manipulations provide a basic form of "bio-technology" that can be readily transferred to both subsistence farmers and commercial producers. Recent research and development projects provide case studies affirming that "bio-technology" need not be restricted to advanced 'omics technologies, with irrigation science delivering both novel fundamental science and improved yields in farmer's fields.

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O2-1

In vitro propagation of box tree (*Buxus hyrcana* Pojark.), an ornamental species under danger of extinction

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Box tree (*Buxus hyrcana* Pojark.), is an ornamental tree and shrub species that has application in various industries. Growth and development of box tree is very slow, its rooting is hard and is under danger of extinction. Thus, the purpose of this research was investigation of the effect of different concentrations of BAP and IBA (0, 0.5, 1, 1.5 and 2 mg l^{-1} form each two) on micropropagation of box tree. The experiment was carried out as factorial based on a randomized complete block design in four replications. Results of the present research showed that the largest number of shoots (6.200/plantlet) was obtained in apical buds explants treated with 1 mg/L BAP along with 0.5 mg/L IBA. Apical bud explants were produced the maximum node number (4.100/plantlet) in medium containing 1 mg/L BAP and the largest number of leaf (with average of 6.566/plantlet) along with 1.5 mg/L IBA. Also, the largest number of root (6.466/plantlet) was calculated in explants treated with 1 mg/L BAP plus 1.5 mg/L IBA. Plantlets were transferred to pots containing peat and perlite with ratio of 1:1 for acclimatization. The pots were kept in a greenhouse with temperature of 24–26 °C and relative humidity of 70% and periodic irrigation. Around 90% of those were healthy. These acclimatized plantlets were similar to mother plants.

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O2-2

Benefits of vitamin B5 regulation on CHO cells energy homeo-stasis and therapeutic production

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Background and novelty: Vitamins are essential micronutri-ents required to support the growth and propagation of any living cell. Indeed, mammalian cells cannot synthesize them, and the lack of vitamins in the diet is directly linked to severe cellular defects [1–3].

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Changes in central metabolism limits growth and recombi-nant protein expression highlighting a regulatory link between cell metabolism, metabolite consumption and accumulation and cell growth [4].

Experimental approach: We designed an improved selection method based on the co-expression of vitamin B5 intracellular transporter, relying on mammalian cell dependence on this vitamin for energy production. We deciphered the molecular and metabolic changes in the resulting B5-selected populations, and chemically engineered recombinant cells with improved energy homeostasis.

Results: The B5-selection method yields polyclonal cell popu-lations producing recombinant proteins at homogeneous and high level, using the selective advantage of improved cell metabolism, growth and viability. This method is also efficient to recover variant cells synthesizing difficult-to-express chimerical proteins at ele-vated levels, unlike state-of-the-art procedures. Understanding the associated metabolic changes led us to produce cells with increased viability and hence recombinant protein productions.

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O2-3

Biological activities, safety evaluation and phytochemical anal-ysis of grasses used in South African traditional medicine

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The Poaceae plant family is mainly grown for crops. How-ever, some grasses possess medicinal properties with potential application in human and livestock health. This study aimed to document and evaluate the biological activities (antibacterial and antioxidant), safety and phytochemical content in grasses used in traditional medicine by Zulu's in three locations in KwaZulu-Natal Province, South Africa. Twelve grass species used for medici-nal purposes amongst the local communities were documented from the survey. The most noteworthy antibacterial activities (MIC, 0.625 mg/ml) were found in *Coix lacryma-jobi* (roots and leaves, hexane) and *Setaria megaphylla* (roots, DCM) extracts against *Staphylococcus aureus*. The highest ferric reducing power was detected in the whole plant extract of *Cynodon dactylon* (0.085 ± 0.45; r^2 = 0.898). Based on the Ames test, all the grass extracts evaluated were not mutagenic. Root methanolic extracts of *Cymbopogon* spp., *Cymbopogon validus* and *Cenchrus ciliaris* con-tained relatively high levels of total phenolics (27-31 mg GAE/g DW) and flavonoids (4–13 mg CE/g DW). Across the evaluated grass species, the most common phenolic compounds contained were

ρ-coumaric, ferulic, salicylic and vanillic acids. Condensed tan-nins and total iridoid content was highest in *Cymbopogon validus* (2.3 mg CCE/g DW, 3.2 mg HE/g DW, respectively). Findings vali-date the use of grasses in traditional medicine and their potential as being sources of valuable secondary metabolites. The phytochem-icals quantified in the grass extracts might be responsible for the observed biological activities. Therefore, the isolation of bioactive compounds and their characterization is under investigation.

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O2-4

CRISPR/Cas9-induced monoallelic mutations in the cytosolic AGPase large subunit gene *APL2* induce the ectopic expression of *APL2* and the corresponding small subunit gene *APS2b* in rice leaves

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The first committed step in the endosperm starch biosyn-thetic pathway is catalyzed by the cytosolic glucose-1-phosphate adenylyl transferase (AGPase) comprising large and small sub-units encoded by the OsAPL2 and OsAPS2b genes, respectively. OsAPL2 is expressed solely in the endosperm so we hypothesized that mutating this gene would block starch biosynthesis in the endosperm without affecting the leaves. We used CRISPR/Cas9 to create two heterozygous mutants, one with a severely truncated and nonfunctional AGPase and the other with a C-terminal struc-tural modification causing a partial loss of activity. Unexpectedly, we observed starch depletion in the leaves of both mutants and a corresponding increase in the level of soluble sugars. This reflected the unanticipated expression of both OsAPL2 and OsAPS2b in the leaves, generating a complete ectopic AGPase in the leaf cytosol, and a corresponding decrease in the expression of the plastidial small subunit OsAPS2a that was only partially complemented by an increase in the expression of OsAPS1. The new cytosolic AGPase was not sufficient to compensate for the loss of plastidial AGPase, most likely because there is no wider starch biosynthesis pathway in the leaf cytosol and because pathway intermediates are not shuttled between the two compartments.

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O2-5

Enzymatic modification for the production of healthy lipids from new omega-3 sources: chia, camelina and echium oilseeds

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Sustainable alternatives sources of omega-3 have awaken increasing interest to provide worldwide demand. Novel plants like chia (*Salvia hispanica* L.), camelina (*Camelina sativa*) or echium (*Echium plantagineum*) contain significant oil amounts with good nutritional value. Omega-3 content for chia oil is 65% α-linolenic acid (ALA), 29% ALA for camelina oil, while echium oil is char-acterized by 35% ALA and 15% stearidonic acid (SDA), which is not common in vegetable oils. In this work, different healthy lipids enriched in omega-3 were synthesized by lipases from *Thermomyces lanuginosa* (TLL), *Candida antartica* B (CAL-B) and *Rhi-zomucour miehei* (RML).

Chia oil was used to produce omega-3 concentrates in form of fatty acid ethyl esters (FAEEs). Immobilized TLL and its improved derivatives allowed high FAEEs synthesis (93%) in only 4 h. Moreover, TLL derivatives showed better stability keeping 100% activity after reutilization cycles in opposition to commercial derivatives.

SDA enrichment from echium oil was done by optimizing reaction conditions to synthesize 2-monoagclyclyerols (2-MAGs). Maximum theoretical amount of 2-MAGs was achieved in 2 h obtaining a product enriched in 24% SDA.

Finally, structured triacylglycerols (STAGs) from camelina oil were produced by transesterification with EPA/DHA FAEEs. Tested lipases showed different selectivity towards EPA and DHA. CAL-B exhibited the highest STAGs yields in short reaction times, 67.9% and 86.6% after 2 and 4 h, respectively.

In conclusion, these results show the potential interest of chia, camelina and echium oils as new omega-3 sources for enzymatic production of healthy lipids and their promising applications in biotechnology and food industries.

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O2-6

Monitoring cell division rates in *Medicago sativa* roots by developmental live cell imaging

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Sustained plant growth and development is supported by spatio-temporal regulation of two fundamental processes, cell division and cell expansion. In roots, cell divisions are restricted to the meristematic region in the root apex. Mitotic division of plant cell is very dynamic process. It is controlled by rearrangements of microtubule arrays such as preprophase band (PPB), mitotic spindle and phragmoplast during karyokinesis and cytokinesis. Quantitative evaluations of cell divisions in plant roots have been performed in several model species, using reconstruction analyses from fixed samples, or imaging of living roots by conventional microscopy methods. Long-term imaging by conventional microscopic methods, however, is compromised by artificial horizontal positioning of plants, limited nutrient supply, pressure between microscopic slides, and high energy illumination leading to undesirable phototoxicity and photodamage. We performed quantitative study of root growth rates and durations of individual mitotic stages in diverse tissues of transgenic *Medicago sativa* roots carrying microtubule marker GFP-MBD. We employed light-sheet fluorescence microscopy (LSFM), a new method providing proper spatio-temporal and physiological live imaging of plant root development. We have found a positive correlation between root growth rate and dynamic pattern of microtubule arrays in dividing cells of growing roots of alfalfa, an important legume crop.

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Symposium 3: Metabolic Engineering and Synthetic Biology

O3-S

RNA aptamers as genetic control devices – the potential of riboswitches as synthetic elements for regulating gene expression

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RNA utilizes many different mechanisms to control gene expression. Among the regulatory elements that respond to external stimuli, riboswitches are a prominent and elegant example. They consist solely of RNA and couple binding of a small molecule ligand to the so-called “aptamer domain” with a conformational change in the downstream “expression platform” which then determines system output. The modular organization of riboswitches and the relative ease with which ligand-binding RNA aptamers can be selected in vitro against almost any molecule have led to the rapid and widespread adoption of engineered riboswitches as artificial genetic control devices in biotechnology and synthetic biology over the past decade.

We will highlight proof-of-principle applications to demonstrate the versatility and robustness of engineered riboswitches in regulating gene expression in bacteria and report about new strategies of synthetic riboswitches in eukarya with a special focus on the control of alternative splicing. We will report on strategies and parameters to identify aptamers that can be integrated into synthetic riboswitches, before finishing with a reflection on how to improve the regulatory properties of engineered riboswitches, so that we cannot only further expand riboswitch applicability, but also fully exploit their potential as control elements in regulating gene expression.

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O3-1

Adaptation of the yeast *Kluyveromyces marxianus* to a biotechnological niche

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Kluyveromyces marxianus is traditionally associated with fermented dairy products, but can also be isolated from diverse non-dairy environments. Because of thermotolerance, rapid growth and other traits, many different strains are being developed for food and industrial applications but there is, as yet, little understanding of the genetic diversity or population genetics of this species. The genomes of several strains have been sequenced in recent years and, in this study, we sequenced a further 9 strains from different origins. Analysis of the Single Nucleotide Polymorphisms (SNPs) in 14 strains was carried out to examine genome structure and genetic diversity. It was found that the isolates include haploid, diploid and triploid strains, as shown by both SNP analysis and flow cytometry. All 6 isolates from dairy environments were diploid or triploid, whereas 6 out 7 isolates from non-dairy environment were haploid. This also correlated with the presence of functional *LAC12* alleles that encode a lactose permease only in dairy haplotypes. The diploids were hybrids between a non-dairy and a dairy haplotype, whereas triploids included 3 copies of a dairy haplotype. These data are consistent with a hypothesis that the natural reservoir of *K. marxianus* is non-dairy and its use to produce fermented milk products has selected for a set of strains with higher ploidy that are adapted for dairy fermentation. Other adaptions for sugar utilisation are also evident in the genome. Detailed molecular analysis using newly-developed genome engineering tools is uncovering the basis of the flexible sugar-utilisation capacity of *K. marxianus*. The implications of this for strain selection and design for uses in biotechnology will be discussed.

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O3-2

16S and 18S metagenomics analysis of fouling residues from aquaculture

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Sea based aquaculture creates an environment optimal for microbial growth. The biofilm generated on the infrastructure is a unique symbiosis of bacteria and small single cell eukaryotes that may influence the fish health and welfare. Few metagenomic studies have utilized both 16S and 18S rRNA targets to identify the composition of the net pen growth. The Ion Torrent PGM platform enables both 16S and 18S metagenomics analysis, which we have utilized to study fouling residues from different aquaculture facilities in North West Norway. For both the 16s and 18s rRNA metagenomics we target all variable regions of the genes to optimize species identification. Net pens are cleaned regularly so we have collected samples before and after cleaning to identify key organisms involved in net pen growth. The results show a highly diverse communities, although Rodobacteraceae are dominating the samples.

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O3-3

Synthetic transport systems in bacteria

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Integration of non-natural compounds into biological processes offers great potential to modify or expand existing cellular functions. Cellular uptake of these compounds, however, is often hindered by the selective permeability of cell membranes. Here, we present versatile solutions to overcome this limitation by covalently attaching natural and non-natural cargo molecules to transport vectors that are efficiently taken up by native transport proteins. Most notably, peptide and sulfonate transporters from *E. coli* are promising entry gates into the cell, as they have exceptionally broad substrate ranges. We demonstrate that attaching different cargo molecules to the carboxyl group of a peptide's glutamate side chain or the sulfonate sulfobutanoic acid allows for uptake via these transporters. To intracellularly release cargo molecules from peptides, we exploited the enzyme γ-glutamyl transferase (GGT), which is known to hydrolyze a wide range of γ-substituted glutamates. For the sulfonate-based transport system, a GGT variant was rationally engineered to efficiently hydrolyze sulfobutanoic acid-cargo constructs. The versatility of these synthetic transport systems was demonstrated by delivering structurally diverse amino acids and non-natural dyes into the cell. Furthermore, the system was applied to discover a novel synthesis pathway towards nicotinic acid from a non-natural precursor. Given the promiscuity of the involved transport proteins and GGT, the synthetic transport systems that we have developed offer a highly generalized solution to overcome limitations in cellular uptake and can hence be considered for a wide range of applications.

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O3-4

Yeast chassis design for production of dicarboxylic acids

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Saccharomyces cerevisiae is a widely used microorganism for industrial biotechnology that has great potential to replace traditional petrochemical synthesis. Optimization of cell factories for production of different biotechnological products is still a cost and time inefficient process. Availability of pre-optimized yeast chassis cells, with improved precursor supply, will overcome such hurdles. Building upon this premise, we have developed a framework for rational design of chassis strains combining genome-scale metabolic models with a multi-objective metaheuristic approach. Here, we present the non-intuitive gene deletion targets optimized for growth-product coupled production of a family of C4-dicarboxylic acids, namely fumaric, succinic and malic acids. Several multi-gene deletion strains, including the chassis cell and the final producer strains, were implemented and experimentally tested. The strains encompassing the chassis backbone produce higher yields of respective targeted compounds than those containing merely the intuitive gene deletion(s). Taking advantage of the growth-product coupled design, best producing strains have been improved by adaptive laboratory evolution. As a proof-of-concept, we have generated pre-optimized chassis yeast cells for enhanced production of C4-dicarboxylic acids, hence showing that modular design strategies may contribute to accelerate cell factory development.

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O3-5

Extracorporeal application of eddy brakes to control the magnetic nanoparticles and modulating the drift and diffusion characteristics of these particles in the heart – a theoretical assessment

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Aims: To develop a novel technique for localization of the multifunctional magnetic nanoparticles in the heart and to modulate the diffusion characteristics in the heart.

Methods: A theoretical method was developed based on the governing equations to control the diffusion and drift of the magnetic nanoparticles. Stroke's–Einstein, Langevin and Maxwell equations were used to study the properties. Dipole–dipole interaction and Brownian motion of the particles were considered.

Results: A model was developed to accumulate the magnetic nanoparticles in the heart. A external magnetic filed (dipole) of 3–6 T placed perpendicularly is used, and a large nitinol grid patch of (12 × 12) square cm with micro-netting of nitinol (50/75 μ) to be placed below the heart superficially on the skin posteriorly. The magnetic field is generated by an electromagnet, which is pulsed and timed to diastolic time interval. The external magnetic field

is placed below the anatomical landmarks of the heart to generate maximal magnetic flux (BA cosθ). The interfacing nitinol grid is magnetizable, and when subjected to a strong magnetic field it generates significant eddy currents, and by adjusting the timing eddy brakes can be applied to accumulate the particles in the heart. The drift velocity and diffusion of the magnetic nanoparticles can be controlled by reducing the particle sizes (n). Addition of a functional group on the particles increases the diffusion of the particles. A magnetic exposure time of about 45 min maximizes the diffusion and drift.

Conclusion: A theoretical model can be used to control the magnetic nanoparticles by extracorporeal method and to adjust the drift and diffusion characteristics of the magnetic nanoparticles in the heart.

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O3-6

Products from methanol by metabolic engineering of *Bacillus methanolicus*

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Industrial biotechnology widely uses microorganisms as biochemical factories for sustainable production of chemicals for industrial and medical applications. The one-carbon (C1) compound methanol is a non-food raw material that has a high potential as an alternative raw material for microbial bioprocesses. The methylotrophic and thermophilic bacterium *Bacillus methanolicus* can grow on methanol as sole carbon and energy source, and this organism has proven to be an interesting host for conversion of methanol into value-added products. The *B. methanolicus* genome sequence, accompanied with biochemical and regulatory characterization of key genes and enzymes involved in methylotrophy and central metabolism, has opened for systems-level metabolic engineering on this bacterium for production of amino acids and platform chemicals. This presentation will describe metabolic engineering of *B. methanolicus* for production of platform chemicals Cadaverine and GABA from methanol.

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O3-7

Naturally occurring novel promoters for recombinant protein production around pyruvate branch-point in *Pichia pastoris*

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Novel strong *Pichia pastoris* promoter discovery is crucial in metabolic engineering for recombinant protein (r-protein) production. Transcriptome and proteome data of *P. pastoris* were analysed and genes having higher expressions than glyceraldehyde-3-phosphate dehydrogenase (GAP) gene under limited-oxygen-transfer conditions were identified as promoter sources. Two promoters around pyruvate-node were determined as promising candidates, and in silico analysis of putative promoter regions was conducted. Promoter of pyruvate kinase (P_{PYK}) has one transcription start-site with a minimum promoter-score of 0.99; while, promoter of pyruvate decarboxylase (P_{PDC}) has two start-sites each having a minimum promoter-score of 0.91. To drive gene expression, putative promoter-regions were replaced with P_{GAP} in parent plasmid pGAPZαA::hGH, harboring human-growth-hormone (hGH) gene. *P. pastoris* strains carrying single-copy hGH under PPYK, PPDC, and for comparison under P_{GAP} were tested in high-cell-density rhGH fermentations abbreviated, respectively, as BRPYK, BRPDC, and BRGAP. Promoter strength was evaluated by mRNA transcription-copy-number (mTCN) results, rhGH concentration measurements, and calculated flux distributions. P_{PDC} and P_{PYK} performed higher activity compared to PGAP. Maximum rhGH production was obtained in BRPDC as 122 mg dm^{-3} at t = 15 h, and then in BRPYK as 101 mg dm^{-3} at t = 12 h, and in BRGAP as 58 mg dm^{-3} at t = 9 h; while, mTCN of hGH was the highest with P_{GAP} at t = 15 h, and then with PPDC with the maximum at t = 12 h, and lowest with P_{PYK}. Flux distributions demonstrated perturbation effects of the naturally-occurring-novel-promoters in the engineered systems and validated the cross-pathway regulatory interactions.

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Symposium 4: Nanomedicine

O4-S

IronMan: moving nanomedicine from lab to market

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Innovation in nanomedicine is mostly led by start-ups and SMEs in Europe, Addressing unmet medical needs, their research and development programmes are delivering innovative nanoformulations for therapeutic, diagnostic or patient monitoring applications in a wide range of diseases. Preclinical proofs of concept are delivered at different stages of maturity. However entrepreneurs are then facing huge challenges in translating their proof of concept to the clinical validation, regulatory approval, industrialisation, manufacturing and finally to the market.

The European Technology Platform on Nanomedicine ETPN has initiated in 2015 the Nanomed Translation Hub which comprises a set of complementary services dedicated to nanomed entrepreneurs. It includes (a) EUNCL the European Nanomedicine Characterisation Laboratory for the full physical, chemical and biological preclinical characterisation of nanophamaceuticals, (b) three pilot lines for upscaling the production of nanobiomaterials before the clinical trials and (c) the TAB Translation Advisory Board to advice, guide and coach entrepreneurs in their strategic choices of industrialisation. All these services are offered for free for European applicants because it is funded under Horizon 2020 Framework Programme. More than 100 applications have been processed so far with huge benefits for applicants.

Further to this first period of implementation of 3 years, the Nanomed Translation Hub is evolving in order to serve nanomed entrepreneurs at an earlier stage of development and thus increases their chances of success.

The success of this translation hub dedicated to nanomedicine paves the way to the widening of the concept to medical technologies at large, under ESTHER European industry driven initiative.

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O4-1

Engineering protein nanocages for targeted photodynamic therapy

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Photodynamic therapy (PDT) is a selective and minimally invasive cancer treatment. To destroy tumour cells, PDT relies on photosensitizers that can be activated by light to convert the oxygen within tumours into highly toxic reactive oxygen species (ROS) that induce cell death. The red fluorescent protein KillerRed is a biological photosensitizer that produces large amounts of ROS upon excitation with green light. In contrast to chemical photosensitizers, KillerRed is water soluble, biocompatible, biodegradable, and can be fused genetically to cancer-targeting proteins for targeted PDT. However, low bioavailability, concentration and localization can reduce the therapeutic effect of KillerRed. To overcome these challenges, we propose the encapsulation of KillerRed within encapsulin nanocages thus providing a reliable platform technology for site-specific PDT. Encapsulins are self-assembling protein nanocages isolated from bacteria, and represent a new class of biological nanoparticles that are biocompatible. Their external surfaces can be genetically modified to display peptides (e.g. cancer-targeting peptides) that mediate cell-targeting. Furthermore, unlike other protein nanocages, encapsulin selectively encapsulates proteins tagged with a unique C-terminal cargo-loading peptide (CLP) during its self-assembly. Herein we present, the production of an engineered encapsulin variant that has been loaded with CLP-tagged KillerRed and also displays cancer-targeting peptides on its outer surface. This will be tested for its ability to target and kill cancer cells both *in vitro* and *in vivo*.

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1871-6784/

O4-2

In situ biosynthesis of bacterial nanocellulose incorporated with hydroxyapatite/cellulose nanocrystals

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Bacterial nanocellulose (BNC) is a biopolymer consisted of fibers network using in medical application due to its biocompatibility and high purity of cellulose. Hydroxyapatite (HA) is an inorganic constituent of natural bones and teeth. This study focused on the production of BNC/HA composites by using cellulose nanocrys-tals (CNCs) with improved the colloidal stability of HA. CNCs were concomitantly added during HA synthesis (HA/CNCs, HC) as HA dispersant. HC was applied in to the culture medium during BNC biosynthesis. Morphology, crystallinity, chemical functional groups and element of BNC/HA/CNCs (BHC) composites were character-ized. Transmission electron microscope (TEM) images showed that HA particles were localized on CNCs structure. The present of HC in BNC structure was confirmed by scanning electron micro-scope (SEM), energy dispersive spectrometer (EDS) and Fourier transform infrared (FT-IR) spectrometer. X-ray diffraction exhib-ited the decrease of crystallinity index of CNCs (70.90 ± 0.76%) and HC (24.25 ± 3.44%). Therefore, these results suggested that the pro-duction of BHC composites by using CNCs assisted dispersibility of HA showed a potential method to prepare BNC as scaffold in tissue engineering.

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O4-3

Study on the antioxidant activity and *in vitro* distribution in mice of gold nano particles/carboxymethyl chitosan synthesized by radiation techniques

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Gold nanoparticles (AuNPs) with sizes about 5.2, 6.7 and 7.3 nm were synthesized by γ-irradiation of 0.25, 0.5 and 1.0 mM Au3+ solutions using carboxylmethyl chitosan as stabilizer. The optical characteristics and particles sizes of AuNPs were determined by UV–vis spectra and TEM images, respectively. The antioxidant activity of AuNPs at various concentrations was investigated using free radical ABTS. The results showed that the higher concentration of AuNPs displayed the stronger antioxidant activity. This activity of AuNPs was also increased by the increase of reaction time and higher than that of ascorbic acid. The synthesized AuNPs were intravenously injected into tail of mice with a dose of 1 mg AuNPs per mouse for investigation of the in vivo distribution of AuNPs at different times. The analytical results showed that the blood haematological and serum biochemical indexes of mice administrated with AuNPs were no significant different from those of the control ones. The gold content in the samples determined by k0-neutron activation analysis (k0-NAA) method indicated that after injection 1 h, AuNPs were mainly accumulated in liver (64.92%), blood (31.33%) and a small amount in lungs (2.16%) and kidneys (1.60%). After 6 h, AuNPs content almost disappeared in blood, but its accumulation was increased in livers (88.85%), lungs (8.55%) and kidneys (2.10%). After 12 h, the content of AuNPs was found to be slightly reduced in liver and almost unchanged in lungs and kidneys. Thus, the above synthesized AuNPs may potentially be applied in cosmetic, functional food and pharmaceutics.

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O4-4

Oral delivery of alternative protein scaffold Nanofitins targeting TNFα shows a strong anti-inflammatory effect in models of inflammatory bowel diseases

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Despite a remarkable efficacy, treatment of inflammatory bowel diseases using systemic administration of anti-TNFα antibodies remains associated to several limitations such as a high cost, immunogenicity, poor patient compliance and side effects related to their systemic exposure. Oral administration of anti-TNFα therapeutics would benefit from better targeting to the site of inflammation in the gut while decreasing their systemic exposure and related side effects. The European SADEL project aimed at developing oral anti-TNFα biotherapeutics based on Nanofitins, a novel alternative protein scaffold.

In this project, the *in vivo* efficacy of the Nanofitins at locally reducing the lesions in mouse models of colitis was considered as reflecting both their stability at the site of the disease and their capacity at neutralizing TNFα. These assays performed in the early phase of the selection cascade allowed finding potent Nanofitin candidates with a high stability profile in the gut, as demonstrated by the evaluation of their stability in simulated biorelevant fluids. Further optimization of their neutralization potency by affinity maturation resulted in an enhanced *in vivo* anti-inflammatory effect. Finally, we demonstrated *in vivo* that the anti-inflammatory action of the Nanofitins was indeed TNFα driven.

In summary, the extreme stability of the natural Nanofitin scaffold allowed the generation of oral biotherapeutics that effectively locally target TNFα reducing the overall inflammation in colitis. Anti-TNFα Nanofitins offer a promising way to alleviate the medical burden for inflammatory bowel diseases.

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O4-5

Green approaches for preparation and comparative analysis of three different molecular sizes of multifunctional sericin nanoparticles

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The sericin nanoparticles were prepared (SNPs) during extraction under the reductive condition at room temperature using 0.02 M sodium carbonate solution and chopped cocoons obtained from *Antheraea mylitta*. SNPs were fractionated by ultrafiltration into three different molecular sizes: fraction 1 (SNP1) contains the proteins ranging from 50–200 kDa, fraction 2 (SNP2) 30–50 kDa and fraction 3 (SNP3) 10–30 kDa. The formations of SNPs were characterized by UV–vis spectroscopy: showing a characteristic hump of nanoparticle formation in 300–400 nm region; zeta potential: confirmed SNP1 was highly stable. The FTIR spectra: confirmed the presence of functional groups; the FE-TEM and FE-SEM images: confirmed the synthesized SNPs were spherical in shape with smooth surfaces. The particles diameter were around 140–200 nm (SNPs), 66–80 nm (SNP1), 30–40 nm (SNP2), 16–20 nm (SNP3). Further, the X-ray diffraction analysis confirmed that SNPs were amorphous in nature. The antioxidant activity of these fractions using different concentrations (200, 100 and 20 μg/ml) of SNPs showed SNP1 is more active than SNP3 and SNP2. Anti-proliferative assay on MDA-MB 231 cells showed that SNP2, SNP3, and SNP1 inhibited cancer cell growth more effectively than normal human keratinocyte (HaCaT) cells. Further, analysis of cell cycle by flow cytometry showed that SNP2 arrested the cell growth in G1 phase. Anti-bacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria showed SNP1 was more potent than SNP3 and SNP2. Overall these data suggest that different SNPs possess different biological activities which can be used accordingly in a specific application.

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O4-6

Adenosine delivery via squalene-adenosine nanoparticles to treat ischemic diseases: liver versus brain

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Even though adenosine is expected as a potent anti-inflammatory medicine, its clinical use has been limited by its very short circulation half-life. This drawback can be overcome by its conjugation to squalene – a natural triterpene – leading to the formation of stable nanoparticles. Recently, our team demonstrated that these squalenoyl adenosine nanoparticles (SQAd NPs) displayed a beneficial effect on cerebral ischemia and spinal cord injury. Moreover, the biodistribution profile of these nanoparticles in mice showed their accumulation in the liver and revealed the release of adenosine in this organ up to 24 h after intravenous injection. As a result, SQAd NPs were tested for hepatoprotection on two different pre-clinical models: global hepatic ischemia and concanavalin A induced hepatic inflammation. Surprisingly, absolutely no protection was observed on either of these pathologies. To understand the difference between brain and liver responses to SQAd NPs, the mechanism of action of this nanomedicine needs to be elucidated. Radioligand displacement studies for quantitative analysis of ligand-receptor interaction did not show any affinity of SQAd NPs for adenosine receptors under relevant conditions (i.e. pre-incubation with serum). Hence, this prodrug can be considered as inactive in this experimental in vitro model. Why SQAd is pharmacologically efficient in neurological injuries but not in liver diseases remains an unanswered question which deserves further investigations. Interestingly, although acute adenosine release has been associated with hepatoprotection in the literature, chronic adenosine overproduction is a known cause of liver pathologies like cirrhosis and fibrosis, which might explain why prolonged adenosine delivery by SQAd NPs in the inflamed liver may be detrimental.

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Symposium 5: Cell Therapy

O5-1

Evaluation of the activity of sophorolipids against microbial biofilms on medical-grade silicone

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Microbial biosurfactants (BS) have recently emerged as a potential new generation of antiadhesive and anti-biofilm agents thanks to their ability to disrupt membranes and to affect the adhesion properties of cells/microorganisms. Consequently, BS medical device coatings can be used as a preventive strategy with enhanced biocompatibility to delay the onset of pathogenic biofilm growth. Among biosurfactant families, glycolipids (sophorolipids, in particular) are relevant molecules for applications in combating many diseases and as therapeutic agents thanks to their antibacterial, antifungal, antiviral and antiadhesive activities. The aim of this project is the evaluation of anti-adhesive and anti-biofilm activity of sophorolipids-coated biomedical materials (i.e. medical-grade silicone and polystyrene as control material) with respect to microbial strains of clinical relevance. In particular, sophorolipids it is been tested for their antimicrobial activity against planktonic and sessile cells of microbial pathogens, as well as, the quantification of the effect of different concentrations of sophorolipids on the adhesion properties and biofilm formation of pathogens was carried out on silicone material in coincubation and pre-coating conditions, at different time-points, showing a significative reduction of the viability of cells in the presence of the tensoactive. This project provides preliminary results for the development of novel functionalization solutions with natural molecules for the prevention of microbial colonization on biomedical devices. These results could bring, in future, to original and innovative achievements with straightforward technological applications in the medical device industry and in clinical setting.

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O5-2

Neurometabolic changes in aging human brain observed with whole brain magnetic resonance spectroscopic imaging

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Aging effects on normal human brain is an important research topic for neuroscience and clinical diagnosis. Knowledge of these effects provides the basis for identification and understanding of pathological neurodegenerative changes. In this project, we demonstrate the usefulness of a recently established whole-brain magnetic resonance spectroscopic imaging (wbMRSI) technique in studying neurometabolic changes in aging human brain, which enables determination of regional metabolite concentrations in multiple brain structures simultaneously, in contrast to conventional magnetic resonance spectroscopy techniques that suffer from limited spatial coverage. The study was approved by local institution review board. Healthy subjects aged between 20 and 70 years ($n \geq 5$ per age-decade for each gender) were studied with magnetic resonance imaging (MRI) and wbMRSI. Regional concentrations of brain metabolites such as N-acetyl-aspartate, choline, total creatine, myo-inositol, glutamine and glutamate, as well as the fractional volumes of brain white matter, gray matter and cerebrospinal fluid were estimated in different brain structures. Our results revealed age-related changes concerning brain gray matter volume and contents of different metabolites in aging human brain. These observed metabolic and microstructural variations with age provide direct in vivo proof that physiological neuronal decline in aging human brain are evident and associated with a reduction of gray matter volume, neuronal density and metabolic activity, in combination with cellular aging indicated by altered cell membrane turn-over, gliosis, and altered energy metabolism. The study showed the high potential for the wbMRSI technique to be a valuable tool used for scientific research and clinical diagnosis on neurodegenerative diseases.

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1871-6784/

O5-3

Nanocomplexes based on chitosan-peptide derivatives towards wound healing promotion

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Dermal wound healing is a complex process, which includes four overlapping steps: inflammation, migration, proliferation and maturation. Although tissue repair often occur spontaneously, it may fail depending on the size and depth of the wound, leading to non-healing, chronic wounds. Therefore there is a growing need for developing biomaterials promoting wound healing in such cases. Wound regeneration needs to be guided by biological cues, such as Arg-Gly-Asp (RGD), a peptide known to induce cell adhesion and migration. Nanocomplexes based on polyelectrolyte self-assembly are suitable carriers for these cues.

Our goal is to develop different formulations of polyelectolyte nanocomplexes for topical wound application: a sprayable suspension of nanocomplexes, nano-structured hydrogels and freeze-dried foams, which would hydrate upon exudate absorption. Formulations are based on the chitosan derivative O-carboxymethyl-N,N,N-trimethyl-chitosan (CMTMC) grafted with RGDC peptide.

Nano-sized polyelectrolyte particles (size about 200 nm) were prepared by complexation of the cationic chitosan derivative with anionic chondroitin sulfate. Hydrogels were obtained by mixing RGDC-functionalized chitosan with hyaluronic acid (HA) at a 1:1 volume ratio. Foams were produced by lyophilization of the previously prepared hydrogels.

Human dermal fibroblasts treated with formulations based on RGDC-derivatized chitosan showed a spread phenotype and increased motility compared to CMTMC. Moreover, these formulations showed *in vitro* to promote wound closure after 24 h. These results were obtained only in presence of the adhesion peptide.

Overall, adhesion peptide-bearing nano-formulations promoted HDF survival, motility and migration. They have the potential to accelerate cell migration *in vivo* and promote healing of chronic wounds.

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O5-4

Targeted cutaneous delivery of an anti-CD29 mAb for the topical treatment of psoriasis

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Integrins are mostly expressed in the basal layer of the epidermis where they are known to be implicated in cell adhesion to the basal lamina. Abnormal expression of integrins is related to a psoriasis-like phenotype and T-cell translocation to the epidermis. OS2966 is a humanized neutralizing anti-CD29 (β1-integrin subunit) monoclonal antibody for the treatment of glioblastoma. Local application of OS2966 and its binding to CD29 could be of therapeutic interest in the treatment of psoriasis and inhibition of T-cell migration to the epidermis. Given their proteic nature, monoclonal antibodies targeting cytokines – even those involved in dermatological conditions – are administered parenterally, leading to a high systemic exposure and immunosuppression, together with low bioavailability at the target site: the skin.

This is the first investigation into the topical cutaneous delivery of OS2966, a 150 kDa humanized anti-CD29 monoclonal antibody, using minimally-invasive P.L.E.A.S.E.® Er:YAG fractional laser ablation. The results demonstrated that needle-free delivery of OS2966 into and across skin was feasible. Above laser fluences of 35.1 J/cm^2, skin deposition and permeation were statistically superior to passive delivery reaching values up to 3.7 ± 1.2 μg/cm^2 at the most aggressive condition. Selective targeting of the skin was achieved since at least 70% of the OS2966 delivered, was delivered locally to the skin, with minimal transdermal permeation. Visualization of fluorescently-labelled OS2966 demonstrated the penetration of the antibody at least as deep as the dermo-epithelial junction, a critical border region where immune cells cross to promote disease progression.

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O5-5

An automated microfluidic perfusion device for adherent cell reprogramming, expansion and culture condition monitoring

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To fulfil the promise of personalised cell therapies it is first necessary to generate clinically viable induced pluripotent stem cells from a patient-specific tissue sample. Current derivation methods are constrained by the challenges of primary cell culture, high cost of transfecting agents and low efficiency. Exquisite control over the cellular microenvironment, the dramatic reduction of reagent volumes and the facility for parallelisation and automation make microfluidics highly suitable to determine optimal conditions for reprogramming. To succeed in this venture the microfluidic device must be capable of efficient transfection and homogeneous culture; i.e. to uniformly deliver reprogramming factors and maintain pluripotency of the derived cells. We present a microfluidic culture device (MFCD) which has been validated for both cell transfection and expansion. In particular, our device offers automated transfection of cells and the non-invasive determination of cell culture characteristics, such as cell density and (specific) oxygen uptake.

To transfect on-chip we automate micro-volume injections into our MFCD using an injection valve and demonstrate its application with mouse embryonic stem cells (mESCs) using an eGFP episomal vector. The expansion of mESCs under fully-defined, homogeneous perfusion conditions was also successfully demonstrated. Time-course profiles for culture confluency and cell density are acquired using novel image processing algorithms and phase contrast microscopy images of the entire culture chamber, without disruption to the cells. Similarly, cellular oxygen kinetics are determined in real time using optical sensors. By combining both analytical approaches we can quantify the specific oxygen uptake rate over the culture time-course.

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O5-6

Gene expression-targeted isoflavone therapy for Huntington's and Alzheimer's diseases

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Huntington's disease (HD) and Alzheimer's disease (AD) are severe neurological disorders for which no effective treatment is currently available. Here we studied effects of genistein (trihydroxyisoflavone) on a HD cellular model, consisting of HEK-293 cells transfected with a plasmid bearing mutant HTT gene, and on the streptozotocin-induced rat model of the sporadic form of AD. We found that both level of mutant huntingtin and number of aggregates were significantly decreased in genistein-treated HD cell model. This led to increased viability of the cells. Autophagy was upregulated while inhibition of lysosomal functions by chloroquine impaired the genistein-mediated degradation of the mutated huntingtin aggregates. Specific pathways of the autophagy induction were tested. In studies on the rat model of AD, we demonstrated that high genistein dose (150 mg/kg/day) can also activate autophagy. Complete degradation of beta-amyloid and hyperphosphorylated tau protein in the brain was observed, while experiments with cell cultures demonstrated that these effects require autophagy stimulation. Unexpectedly, behavior of high dose genistein-treated AD rats was indistinguishable from that of healthy animals. Hence, we conclude that through stimulating autophagy, genistein removes the major pathogenic factors of HD and AD. Prolonged induction of autophagy was suspected previously to be risky due to putative adverse effects, however, genistein has been demonstrated recently to be safe and suitable for long-term therapies even at high doses. Therefore, results presented in this report provide a basis for the use of genistein in further studies on development of the potential treatment of HD and AD.

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Symposium 6: Plant Stress Tolerance

O6-S

Plant biotechnology employing signalling and cytoskeletal proteins

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Signal transduction by mitogen-activated protein kinases (MAPKs) and cytoskeletal remodelling represent two key mechanisms involved in the plant development and in the plant response to various environmental factors. MAPKs can regulate transcription factors and cytoskeletal proteins during developmental or stress-related reprogramming of cells and whole plants. Examples of selected MAPK members and cytoskeletal proteins (e.g. katanin, MAP65-1 and EB1c) in diverse plant species (Arabidopsis, barley and alfalafa) will be provided and discussed in the light of their potential use in the plant biotechnology. In addition, new methodological approaches including genetic engineering and advanced microscopy methods such as super-resolution and light-sheet microscopy for more precise gene regulation and spatio-temporal visualization of MAPKs and cytoskeletal proteins will be highlighted. Supported by National Program for Sustainability I (grant no. LO1204) by the Czech Ministry of Education, Youth and Sports.

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O6-1

Development of stomata is mediated by interactions between heat shock proteins 90 and YODA signalling pathway

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Stomata are small cellular pores located in the epidermis of plants accommodating gas exchange and water vapor. Stomatal ontogenesis is considered crucial for plant development and environmental adaptation since it contributes into the optimization of photosynthetic efficiency and water management. Environmental parameters such as light intensity, humidity, and carbon dioxide concentrations, as well as internal cues including developmental programs and hormones, influence stomatal guard-cell formation and the distribution of stomatal complexes in the leaf epidermis. Stomata development requires a ligand–receptor module, mitogen-activated protein kinase (MAPK) signalling cascades and nuclear transcription factors. MAPK pathways integrate the signal from the ligand–receptor module transducing it to the transcription factors regulating stomatal development and patterning. Heat shock protein 90 (HSP90) is a highly conserved molecular chaperone modulating a broad range of signalling pathways via interactions with a plethora of client proteins. Here we report a new function of HSP90s in combination with YODA signalling pathway in stomatal lineage specification by providing genetic, biochemical and molecular evidence. We monitored changes in stomatal patterning, distribution and morphology considering physical and genetic interactions between HSP90s and YODA. Moreover, we show that HSP90s facilitate MAPK3/6 activation/phosphorylation and subcellular distribution, while they modulate the transcriptional and protein abundance of downstream targets such as transcription factors SPCH, MUTE and FAMA. We therefore suggest that HSP90s mediate stomatal development via modulation of the function and the abundance of individual components of the YODA signalling pathway.

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O6-2

Evaluation of plant growth promoting rhizobacteria activities of microorganisms isolated from natural sources

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The current trend of reducing the use of synthetic fertilizers leads to the research of new biotechnological products based on microorganisms that enhance plant growth and increase their productivity.

The PGPR (Plant Growth Promoting Rhizobacteria) are microorganisms that are usually found in soils and have the capacity to increase the development in plants. It could be either directly, by providing nutrients to the roots or indirectly, by contributing to increase resistance to pathogens.

To isolate this type of microorganisms and screen them from the rest of the microbiota present in the soil, it is necessary to evaluate the activity that they could perform in benefit of the crops.

To study the biostimulant capacities, it is required to analyze several factors that define the function of each microorganism in the plant ecosystem. Some of these factors are the production of plant hormones such as indoleacetic acid, the production of enzymes such as proteases, the phosphate solubilization ability, the production of siderophores, the fixation of environmental nitrogen and/or the 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity among others.

In this work, the isolation of new microorganisms that combine different characteristics from PGPR from immediate environmental sources has been done. The isolation has been carried out using selective medium cultures to obtain strains that show at least one potential activity to increase plant growth. Once the isolation was done, all the strains were evaluated for seven PGPR characteristics, showing the best candidates which present several PGPR properties.

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O6-3

Multi-tasking of SERK-like kinases: current status and biotechnological advances

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In plants, exogenous signals play a vital role in cell metabolism modification leading to growth and defense responses. The SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) belongs to a family of leucine-rich repeat receptor-like kinases (LRR-RLKs), involved in cell to embryo transition, plant environmental response and plant development. SERKs associate with multiple ligand-binding receptors with complex signalling networks and appear to function in diverse biological processes in plant development and physiology. The present review explores the current status of the role of SERK genes as candidate markers during plant embryogenesis. Furthermore, we review recent advances in newly identified SERK functions and provide novel insights into different biotechnological advances.

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O6-4

Withdrawn

O6-5

New regulatory roles of phospholipase Da1 in *Arabidopsis* as revealed by shot-gun proteomic analysis

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Phospholipase D alpha 1 (PLDα1) is a phospholipid hydrolyzing enzyme playing multiple regulatory roles in plant stress responses. Signalling activity is mediated mainly via the PLDα1 dependent phosphatidic acid (PA) production and by the capacity of PLDα1 to bind and modulate G-protein complexes. PLDα1 and PA are involved in stomatal closure and plant responses to dehydration, salinity and cold stress. PA also modulates actin and microtubule organization. Here we present a comparative shot-gun proteomic analysis of two t-DNA insertion Arabidopsis mutants *plda1-1* and

plda1-2. A significant part (almost 30%) of differentially regulated proteins in both *plda1* mutants (as compared to the wild type) are implicated in metabolism and RNA binding. The latter functional class involves proteins related to translation, RNA editing, processing, stability and decay. Interestingly, several proteins share a common function in chloroplast biogenesis and leaf variegation, a phenomenon not described in *plda1* mutants so far. This is accompanied by alterations of proteins involved in chloroplast protein import. Other important features of plda1 mutants are impaired imports of mitochondrial and nuclear proteins as well as disturbed cell wall architecture and redox homeostasis. Our data also points to the important role of PLDα1 in endomembrane transport. We suggest that PLDα1 is required for membrane fusion events and clathrin assembly. This study helped to uncover new functions of PLDα1 in plants, and broadened potential biotechnological applications.

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O6-6

Stabilization of endangered heritage objects: call a microbial plumber!

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We aim to provide sustainable solutions for heritage using ecologically friendly biological treatments. These methods are based on the development of bacterial extraction methods of iron species from waterlogged wood or on the formation of passivating biogenic layers that can be applied for preserving copper- and iron-based heritage, in particular sculptures but also archaeological objects.

So far, the methods used are often unsatisfactory in terms of efficiency and durability. Also, inhibitors or complexing agents are toxic and pose potential threats to human health and to the environment. Taking advantage of unique properties of microorganisms, reactive corrosion products are extracted or converted into biogenic compounds that provide the treated surfaces with long-term stability.

While often considered as harmful for cultural heritage, microorganisms can also be used for its safeguarding. Over the last decades, the development of biological methods and materials became a significant alternative for the preservation of ancient heritage. In particular, microbial mechanisms are exploited aiming to consolidate, clean, stabilize or even protect surfaces of cultural items.

Data resulting from our research aiming to the preservation of copper- and iron-based artefacts as well as waterlogged wood will be presented. In particular, the application of biopassivation processes on different alloys resulted in the formation of biogenic layers those performances are assessed through stratigraphy studies and/or ageing procedures. Not only copper-based alloys were successfully treated but also iron, zinc and aluminum alloys that are commonly found as architectural parts. In parallel extraction methods based on siderophores or specific bacteria are developed to remove iron and sulfur from waterlogged wood. Protocols to provide mock-ups samples closed to real artefacts are proposed.

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Symposium 7: Fermentation and Downstream Processing

O7-1

BioProcess optimization through yeast-derived nutrients care-ful selection is key for probiotics and recombinant proteins industrial manufacturing

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Probiotics are microorganisms that exhibit a beneficial effect on the host health. After early researches and clinical studies one of the most challenging questions is how to industrially manufac-ture the probiotics in a way to preserve cells fitness and activity. Most of the candidate probiotics are well-known for their complex growth requirements and even more those originating from human microbiome. The same findings can be applied for recombinant protein production using preferred workhorses such us Escherichia coli and Pichia pastoris. Widely used in culture media, yeast extracts and peptones (YE) are a natural source of peptides, amino acids, microelements and other unique nutrients that are pivotal in culture media composition.

As the global leader for yeast-derived products Procelys pro-vides efficient nutrients for the fermentation industry. In response to the rising demand for high quality consistent ingredients, Pro-celys Labs have developed bioperformance assays to investigate YE influence for (industrial) culture media. This work therefore aims atevaluating the ability of selected YE to boost probiotics growth, via-bility and vitality as well as enhancing recombinant protein yield. Using selected models, experiments were performed at small scale and in bioreactors. In each case the key bioprocess drivers were identified and optimized followed by a successful model develop-ment linking YE composition and production performance.

The use of Procelys products allows reaching highly active biomass and protein yield while maintaining cell fitness. In addi-tion, our results demonstrate an accurate nutrients selection procedure is critical for producing highly active probiotic strains and recombinant proteins.

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O7-2

Bacteria as cell factories for producing selenium nanoparticles: their synthesis by the rhizobacterium *Azospirillum brasilense* and characterisation

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Microbial synthesis of nanoparticles (NPs) is of importance for biotechnology linking it to nanotechnology. This process related to “green chemistry” has, however, some problems, e.g. polydispersity of NPs and their low growth rates. Thus, optimising NPs synthesis conditions is necessary for commercial applications. We developed a simple methodology for biogenic synthesis of extracellular relatively uniform spheric selenium NPs (SeNPs) by using bacterial cells (*Azospirillum brasilense* strains Sp245 or Sp7 grown up to the end of the logarithmic growth phase, washed from culture medium and incubated for 24 h with selenite, SeO_3^{2-}), as well as a scheme for SeNPs purification. The size of the SeNPs (~45, . . ., ~90 nm in diameter) depended on the initial selenite concentration (50, . . ., 10 mM, respectively). The SeNPs obtained were characterised by transmission electron microscopy (TEM), dynamic light scattering (DLS) and UV–vis spectrophotometry. Their zeta potential was found to be negative (−21 to 24 mV). The SeNPs sizes determined by DLS and TEM were similar. They were also analysed using Fourier-transform infrared (FTIR) spectroscopy and Raman spectroscopy, which provided complementary information on their composition and structure. FTIR spectra of SeNPs showed the presence of proteins (major components), polysaccharides and lipids. This bioorganic ‘coating’ is their main difference from chemically obtained SeNPs. Raman spectroscopy provided evidence that the SeNPs consisted of amorphous selenium. The SeNPs obtained were also studied by immunochemical analysis (for characterising the bioorganic components). The proposed methodology is promising for nanobiotechnological applications. (Supported in part by The Russian Foundation for Basic Research, Grant 16-08-01302-a.)

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O7-3

Orthogonal, light-inducible protein expression platform in yeast *Sacchararomyces cerevisiae*

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The project Cell2Fab aims at the generation of yeast artificial chromosomes for next generation biotechnological procedures. Fundamental goal is the allocation of modular-build circular YACs that can be easily adapted to different biotechnological applications by facilitating highly regulated production of small to large cohorts of proteins and peptides in the yeast *Saccharomyces cerevisiae*. The long-term goal is the use of YACs for the generation of "multi-enzyme-machines" for the production of pharmacologic relevant peptides, entirely new functional units for next generation biotechnological procedures or the production of proteins for in-vitro-translation. First results for non-conventional cloning technologies and light-induced protein expression for easy and fast construct assembly and protein expression will be presented. We are very interested in meeting other people in synthetic biology and biotechnology, academia as well as industry, to find cooperation partners that are in need of cloning and expression strategies to enable their research and production.

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O7-4

Protein purification using aqueous micellar two-phase systems within a microfluidic device

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Microfluidic devices were recently found as promising tools for liquid–liquid extractions, offering among others continuous process operation and precisely controlled conditions. Higher yields together with reduced consumption of resources and solvents as compared to macroscale systems were achieved, primarily due to smaller dimensions resulting also in reduced distances for transport to the interface and a very large surface-to-volume ratio. Aqueous micellar two-phase systems (AMTPSs) present cheap and environment-friendly alternative to conventional purification methods, enabling high efficiency and good preservation of biomolecules' activity.

The aim of this research was to introduce AMTPS for R-phycoerythrin purification from red algae aqueous extract using a system of microfluidic devices. R-phycoerythrin is a protein found in marine red algae and acts as an auxiliary photosynthetic pigment. It is mainly used as a fluorescence-based marker for numerous molecules in cell biology and immunology, and is also applied as a natural food colorant.

Selective R-phycoerythrin purification from the extract of lyophilised red algae was performed in a microflow system with channels thermostated at various temperatures, enabling the formation of two phases. Phases were separated at the exit of the microchannel by consecutively linked microsettler. The results revealed that the selective fractionation using AMTPS in a microfluidic device was not just quicker, but also more efficient as compared to the conventional batch process. Furthermore, various process conditions could be screened within very short time using small material consumption.

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O7-5

Microparticle-enhanced cultivation of filamentous fungi – insight into the action of microparticles towards various fungal species

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Filamentous fungi are important producers of metabolites and enzymes. The key factor influencing their productivity and the overall run of the bioreactor process is the morphological development of mycelium. Dependent on species and culture conditions fungal morphology is either dispersed (unbranched, branched hyphae, clumps and micropellets) or pelleted (macropellets). Large macropellets are believed to be the ineffective form of mycelium.

One needs to control fungal morphology in the submerged bioreactor cultures and microparticle-enhanced cultivation (MPEC) belongs to the latest and most effective tools. Mineral microparticles greatly affect these fungal species, whose pellet formation mechanism is spore aggregation like at *Aspergilli*. But little is known about microparticles action towards other fungal species of non-aggregative or hyphal aggregation mechanisms of pellet formation.

Thus the initial stages of development (from spores to hyphae) of *Aspergillus terreus*, *Chaetomium globosum*, *Penicillium rubens* and *Mucor racemosus* were quantitatively studied in the standard cultivation and MPEC in shake flasks and bioreactor with the use of image analysis. The action of aluminium oxide microparticles towards these fungi differed much. Expectedly, the microparticles decreased pellet size at spore agglomerating *A. terreus* but they induced pellet formation at non-agglomerating *M. racemosus* normally growing as dispersed mycelium. They caused the formation of core-shell pellets at hyphae aggregating *Ch. globosum* and astonishingly exerted little effect towards *P. rubens*.

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To conclude, the action of microparticles is not as simple as it was previously thought at frequently studied spore agglomerating fungi. Thus, there is a need of the individual approach to each fungal culture, especially when the fungus exerts other than spore agglomerating mechanism of pellet formation or this mechanism is unknown.

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O7-6

Understanding product toxicity in industrial biotechnology

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A major problem in industrial giotechnology is the toxicity of chemical products towards the producing microorganisms, since this restricts product titres and, therefore, limits the uptake of bio-based chemicals manufacturing. An understanding of chemicals toxicity has been obtained by examining the effects of exogenously added chemicals. However, the topology of exposure to the chemicals is different under manufacturing conditions, since the toxic products are formed endogenously. We have therefore set about investigating the effect of chemicals toxicity under production conditions, by analysing chemical stress at a global level in a manufacturing set-up, using a multi-omics approach.

Here we present our first multi-omics datasets from two chemical targets, citramalate and styrene, produced in *Escherichia coli*. Citramalate is relatively non-toxic and was produced to a final concentration of 22.5 g/L. Transcriptomic, proteomic and lipidomic analysis showed that citramalate had very little effect on cellular physiology, with almost no induction of stress responses and no significant changes in membrane lipid composition compared to a non-producing strain. In contrast, styrene was remarkably toxic, and production stopped due to cell death when the styrene concentration reached only 120 mg/L. Significant differences were observed in the regulation of genes and proteins, with some attributable to general stress and some suggesting styrene-specific effects. All data were analysed using DETOXbase, an online tool for integration and analysis of omics and fermentation data. The next step will be to test the hypotheses about the effect of styrene on the cells by using reverse engineering to construct styrene-resistant host strains.

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Symposium 8: Biosensors and Biochips

O8-S

S-layer protein-based biosensors

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Combining biological with electronical components is a challenging approach because it allows the design of ultra-small biosensors with unsurpassed specificity and sensitivity. In particular, self-assembling biomolecules attract great attention as surfaces and interfaces can easily be functionalized and patterned in a bottom-up approach. In this context, crystalline cell surface layer (S-layer) proteins, which constitute the outermost cell envelope structure of bacteria and archaea, are very promising and versatile components in the fabrication of biosensors. S-layer proteins show the ability to self-assemble in-vitro on many surfaces and interfaces to form a crystalline two-dimensional protein lattice. The S-layer lattice on the surface of a biosensor becomes part of the interface architecture linking the bioreceptor to the transducer interface, which may cause signal amplification. The S-layer lattice as ultrathin, highly porous structure with functional groups in a well-defined spatial distribution and orientation and an overall anti-fouling characteristics can significantly raise the limit in terms of variety and ease of bioreceptor immobilization, compactness and alignment of molecule arrangement, specificity, and sensitivity. Moreover, mimicking the supramolecular building principle of archaeal cell envelopes, comprising of a plasma membrane and an attached S-layer lattice allow the fabrication of S-layer supported lipid membranes. In the latter, membrane-active peptides and membrane proteins can be reconstituted and utilized as highly sensitive bioreceptors. This presentation summarizes examples for the successful implementation of bacterial S-layer protein lattices on biosensor surfaces in order to give a broad overview on the application potential of these bioinspired S-layer protein-based biosensors.

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O8-1

Noninvasive prenatal diagnosis by circulating fetal nucleated red blood cells and trophoblasts using silicon-based nanostructured microfluidics

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Background: Noninvasive prenatal testing (NIPT) based on cell-free DNA in maternal circulation has been accepted worldwide but limitations exist, which preclude its full replacement of invasive prenatal diagnosis. We present a novel silicon-based nanostructured microfluidics platform named as "Cell Reveal^{TM}" to demonstrate the feasibility of capturing circulating fetal nucleated red blood cells (fnRBC) and extravillous cytotrophoblasts (EVT) for cell-based noninvasive prenatal diagnosis (cbNIPD).

Methods: The "Cell Reveal^{TM}" system is a silicon-based, nanostructured microfluidics using immunoaffinity to capture the trophoblasts and the nucleated RBC (nRBC) with specific antibodies. The automated computer analysis software was used to identify the targeted cells through additional immunostaining of the corresponding antigens. The identified cells were retrieved automatically for whole genome amplification for subsequent investigations by micromanipulation in one microchip, and fluorescence in situ hybridization (FISH) in another microchip. The captured cells were analyzed with FISH, short tandem repeat analyses, array comparative genomic hybridization, and next generation sequencing, whereas the laboratory is blind to the fetal genetic complement. The cases enrolled in this pilot series include normal control, fetal trisomies, and de novo fetal microdeletions. We even included one dizygotic twin with one de novo microdeletion case.

Results: The genetic investigations confirmed and verified the captured cells to be fetal origin. In every 8 ml of the maternal blood being blindly tested, both fnRBC and EVT were always captured. Our technology can successfully differentiate each cotwin in dizygotic twin pregnancy.

1871-6784/

Conclusions: This report is one of the first to verify the capture of fnRBC in addition to EVT. The scalability of our automated system made us one step closer toward the goal of in vitro diagnostics.

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O8-2

Bacterial bioreporters for standoff detection of buried landmines: molecular manipulations for performance enhancement

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The relative ease by which molecular sensing and reporting elements can be fused together in microbial whole-cell biosensors to generate dose-dependent quantifiable physical (luminescent, fluorescent, colorimetric, electrochemical) responses to predetermined conditions allows the construction of diverse classes of sensors. Over the last two decades we and others have employed this principle to design and construct microbial bioreporter strains for the sensitive detection of either (a) specific chemicals of environmental concern (e.g. trinitrotoluene), or groups of compounds sharing either (b) chemical characteristics (e.g. heavy metals, halogenated organics, etc.) or (c) global biological effects on living systems (e.g. toxicity or genotoxicity). In many of these cases, additional molecular manipulations beyond the initial simplistic sensor-reporter fusion may be highly beneficial for enhancing the performance of the engineered sensor systems.

We have recently described the remote detection of buried landmines using alginate-encapsulated fluorescent microbial (*Escherichia coli*) bioreporters (Belkin et al., 2017, Nature Biotechnol. 35: 308–310). Using this application as a case study, several of the approaches we have adopted over the years to enhance bioreporters' performance will be highlighted. These include random mutations induced in a "directed evolution" process, splitting of the lux reporter cassette, introduction of viral "amplifiers", manipulation of host permeability and the integration of quorum sensing elements.

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O8-3

Development and characterisation of a new fluorescence sensor for online monitoring of bioprocesses

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To meet the high regulatory and quality requirements of the food and drug administration (FDA) for the production of pharmaceuticals, the pharmaceutical and biotechnological companies are encouraged to set up innovative tools to better understand their processes and to ensure batch-to-batch reproducibility. Therefore on-line measurements of bioprocesses are becoming increasingly important.

Fluorescence spectroscopy is a highly sensitive and non-invasive technique for identification of characteristic process states or for the on-line monitoring of substrate and product concentrations. Nevertheless fluorescence sensors are mainly used in academic studies and are not well implemented in monitoring of industrial productions. In this work, we present a newly developed function model of a fluorescence sensor that facilitates the analysis of fluorescence measurements. The setup of the sensor was miniaturised and reduced to measure three important biologic fluorophores (tryptophan, NADH and FAD/FMN) resulting in a significant data reduction. To meet industrial requirements regarding robustness it was furthermore constructed without any moveable parts. The evaluation of the sensor was performed by monitoring batch cultivations of wildtype *E. coli* K1 in a 10L bioreactor scale. The recorded fluorescence data was successfully used to predict the biomass of an independent cultivation. Further possible applications, e.g. the monitoring of eukaryotic cultivations and enzymatic reactions, are currently under investigation.

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O8-4

Highly sensitive aptamer-based nanobiosensors for detection of periodontal disease biomarker

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Periodontitis is a considerable public health problem in most of the countries. According to statistics, eight out of ten people mainly who are age over forty suffer from the dental problem, and there is a high correlation between the periodontal diseases and increased risks for many systemic complications, such as diabetes and cardiovascular diseases. Even though most of the illnesses preventable, the number of patients is still growing since it is hard to get treatments at a proper time. It is because not only there is no pain in its early stage of diseases, but the current diagnosis method can just judge the occurrence of the diseases, not the discerning real-time severity of them, which makes patients' symptom worse. Aptamers that specifically bind to the periodontal biomarker protein molecule were successfully screened out by using immobilization-free Graphene Oxide (GO)-SELEX. Fluorescence Resonance Energy Transfer (FRET) and Surface Plasmon Resonance (SPR) assays were done as follows for analyzing the specificity and the affinity of aptamers to their target. In addition, the pair of aptamers for this biomarker has been successfully applied to lateral flow assay. Finally, these aptamer-based biosensors are applied to real human saliva samples. By monitoring the level of biomarker protein by obtaining the specimen in a non-invasive way, these biosensors can be quickly applied in the early diagnosis of the dental disease.

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O8-5

Detection of endocrine-disrupting compounds by novel yeast biosensors

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We describe the molecular engineering of recombinant Saccharomyces cerevisiae sensor strains for the detection of endocrine-disrupting chemicals (EDC's), and their coupling to high-performance thin layer chromatography (HPTLC) for the simultaneous screening of diverse environmental pollutants.

The presence of EDC's in wastewater and surface, ground and even drinking water is a major concern to public health. The steroid receptors, such as estrogen receptor α (ERα) and the androgen receptor (AR), are part of the nuclear receptor superfamily classified as transcription factors in eukaryotes. The yeast *S. cerevisiae* provides a well defined eukaryotic system for the expression of genes from other organisms. While yeast cells do not contain endogenous steroid receptors, the mammalian steroid receptors demonstrate same function as in mammalian cells, as steroid dependent transcription activators. As a result, yeast can be used as a powerful tool for detecting endocrine disrupting compounds. There exists a variety of yeast-based luminescent, fluorescent or enzymatic assays for the detection of the endocrine-disrupting compounds. These assays, however, are specific and are limited to one group of disrupting compounds per assay. We aim to develop an innovative technological platform for multidimensional detection of EDC's.

For this purpose, we have constructed novel recombinant yeast strains that express either the human ERaor AR, and respond by the synthesis of a fluorescent protein, to the presence of estrogen-like or testosterone-like compounds, respectively. Green, red or blue fluorescent proteins are produced following the binding of the receptor-ligand complexes to the specific hormone receptor element, thereby allowing to differentiate between different biological effects. By coupling this biological assay with HPTLC, a wide variety of compounds can be screened simultaneously.

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O8-6

Improving a bacterial pyranose 2-oxidase using a combination of rational design and directed evolution for biosensor applications

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Modern biosensors can be miniaturized, mass produced and easily transported. Current development is focused on autonomous biosensors for direct on-the-spot measurements. Pyranose 2-oxidases (P2Oxs) can be used as a biosensor for glycemic control in diabetes mellitus, with improved sensitivity as compared with current devices. P2Oxs are flavoenzymes that catalyze the oxidation of D-glucose at the C2 with concomitant reduction of O_2 to H_2O_2. Their lack of anomer preference makes these biocatalysts the perfect candidates to substitute glucose oxidase with improved enzyme specificity. In this work, protein engineering approaches were followed to improve the efficiency of the enzyme AsP_2O_x from *Arthrobacter siccitolerans* towards their successful application in cost-effective portable biosensor devices. This enzyme, the first from bacterial origin, was recently characterized in our lab [1]. In a first step, high-throughput mutagenesis and activity screening protocols were validated and optimized. Next, a library of 25,000 variants was screened using an 'activity-on-plate' assay. From this, a hit was identified showing 5-fold higher specificity as compared to the wild-type enzyme. In parallel, a variant constructed by site-directed mutagenesis, based on structural differences as compared with fungal counterparts, showed a ~4-fold enhancement in the D-glucose specificity. The combination of the mutations derived from the evolution and rational design approaches resulted in a hit variant with a catalytic efficiency 10-fold higher than the wild-type. Work is in progress to further improve the stability and catalytic efficiency of AsP_2O_x toward the construction of a sensing device for glucose detection.

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O8-7

Versatile on-stage microfluidic system for long term cell culture, micromanipulation and time lapse assays

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We report here a versatile on-stage microfluidic cell culture and assay system which is compatible with different microscopes and sensors, can simultaneously perform steps of long term cell culture, high throughput time lapse cell assays/imaging, and cell micromanipulations. With the system, we cultured a variety of cells for different periods of time and monitored their cell morphology, migration and division. We also performed a series non-invasive real time in situ time lapse assays and micromanipulations on different cells. They include: the first time lapse imaging and measurements on the instantaneous variations of morphology, biomechanical properties and the intracellular protein of human red blood cells in responding to pH fluctuation, drug action and electromagnetic radiation; the first continuous time lapse Raman micro-spectroscopy on a CHO cell in different phases of its entire life cycles; the micro-transfection of GFP into B16 cells and the follow up observation of the cell's morphology and expressed GFP fluorescence varying with incubation time and cell generations. The performance of these experiments not only demonstrated the capability of the system, but also proposed a variety of novel methods for obtaining time- and spatially-resolved information about the cellular and molecular heterogeneity and transformation during development or stimulations.

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Symposium 9: New Biotherapeutic Formats

O9-1

Targeted delivery of RNA aptamers to diseased cells

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Huntington's disease (HD) is caused by CAG repeat expansion (encoding an elongated polyglutamine stretch) in the first exon of the *IT-15* gene, forming misfolded and aggregated mutant huntingtin (mhtt). Protein aggregation correlates with disease progression. Several small molecules, antibodies and oligonucleotides have been employed to inhibit the aggregation of mhtt, resulting in improved cell survival. Inhibition of protein–protein interaction and thus, aggregation of huntingtin, could help design a treatment strategy. Nucleic acid aptamers recognize their targets with high affinity and specificity. They are potential agents to inhibit protein-protein interaction and protein aggregation. We report that *in vitro* iterative screening led to the selection of RNA sequences with very high affinity for N-terminal mhtt (N-mhtt). Yeast cells, coexpressing N-mhtt and RNA aptamers, showed protein solubilisation, decreased oxidative stress and increased cell survival. Intracellular aptamers led to alleviation of mitochondrial dysfunction, a key mechanism of disease pathogenesis. Mutant huntingtin-mediated mitochondrial loss, depletion of mitochondrial DNA, decreased ATP production and metabolic activity were rescued by aptamers. Thus, treatment with RNA aptamer(s) is a promising strategy to develop a treatment regime for HD. As oxidative stress is significantly higher in cells expressing mhtt, oxidative stress-inducible aptamer expression vectors were constructed by cloning aptamers under *Thioredoxin 2* (*Trx2*) promoter. Expression of aptamers was significantly higher in cells expressing N-mhtt with concomitant benefit for the cellular phenotype. No change was seen in cells expressing N-wthtt. Thus, this approach can be utilized for targeted expression of nucleic acid therapeutics in diseased cells with high oxidative stress.

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O9-2

Bioinformatics evaluation of amastin protein variants in *Trypanosoma cruzi* for finding conserved T and B cell epitopes for vaccine design

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Trypanosoma cruzi, the causative agent of Chagas disease is a major kinetoplastid protozoan parasite in Latin America. The estimated infected people are about 8 million with annual death toll of 10,000. With no vaccine available for the disease and considering the serious health problems it causes it becomes imperative to design a vaccine against this infection. In the current study Amastin, one of the major surface proteins of the parasite has been used for determining epitopes. From NCBI Protein database 298 amastin protein sequences were obtained and used for multiple sequence alignment for finding out the conserved regions. The variability in the sequences was masked to generate conserved areas. These conserved areas were used for T and B cell epitope prediction. For Tc cell epitopes NetMHCPan, NetCTL, NetChop servers were used whereas for TH cell epitopes NetMHCIIPan server was used. The structures of epitopes for T cells were generated by PEP-FOLD server and HLA allele structures were obtained from Protein Data Bank (PDB) database. The docking to check the binding of epitopes in the cleft of MHC molecules was carried out by Z dock server. B cell epitopes were obtained by BCPREDS and ABCPRED methods. Applying filters like no similarity with human sequences, non-toxin, non-allergen, population coverage analysis and conservancy in more than 80% sequence variants generated six epitopes for Tc cells, two epitopes for TH cells and three epitopes for B cells. The epitopes generated in the study have the potential use for designing future peptide or DNA vaccines.

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1871-6784/

O9-3

Production of antibody-fragments with plasmid-based and genome-integrated T7 *E. coli* expression systems – evaluation of systems performance in microtiter fed-batch-like cultivations

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Although *Escherichia coli* is the most prominent bacterial production host for recombinant proteins, some proteins with high economic potential can still hardly be produced at remunerative levels. We selected four different Fabs (Fragment, antigen binding) (BIBH1, BIWA4, CIMZIA and FabX) with identical constant domains representing such challenging proteins. Fab yield can be affected by miss-folding, aggregation or unbalanced expression, translation and translocation levels of sub-units, making it still challenging to efficiently design expression systems and production processes.

For translocation to the periplasm a post-translational (ompA) and a co-translational (dsbA) leader sequence were used. *E. coli* BL21(DE3) and *E. coli* HMS174(DE3) were transformed either via pET vectors or genome integration. The resulting 32 clones, were cultivated under fed-batch-like conditions in the BioLector.

Cell growth was not affected by leader/Fab combinations but yield of correctly folded Fab ranged from 0 to 12.5 mg/gCDM. Higher expression rates caused higher amounts of free light chain and K12 strain reached higher yields. Except of CIMZIA with the dsbA leader, genome integrated versions showed higher Fab yields, reduced levels of free light chain and basal expression than plasmid-based systems. Independent from used expression system, highest yields were obtained with CIMZIA followed by BIWA4, BIBH1 and FabX. Leader sequence cleavage-efficiency for DsbA was significantly lower than for OmpA, both showed lowest with CIMZIA.

Summarizing, we showed that the selected set of host/gene dosage/leader/Fab combinations resulted in a broad range of variation in terms of Fab yields and processing and will be studied detailed in bench-scale fermentations.

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O9-4

Spy and Snoop protein superglues for nano-assembly and tickling the immune system

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A special feature of the bacterium *Streptococcus pyogenes* enables spontaneous isopeptide bond formation within particular proteins. We have re-engineered this chemistry to generate an irreversible peptide–protein interaction (SpyTag/SpyCatcher). This reaction is rapid, genetically-encodable and specific in diverse biological environments. Cyclizing enzymes using SpyTag conferred resilience to boiling, as may be applicable for biotransformation and enhancing animal nutrition. SpyTag and its related superglue SnoopTag allow programmable synthesis of multi-functional teams, for synergy in control of cancer cell signalling. Virus-like particles (VLPs) are nano-assemblies with many attractive features for vaccination. However, decorating VLPs with target antigens by genetic fusion or chemical modification is often unsuccessful. We demonstrated 100% reaction to SpyCatcher-VLPs after mixing with SpyTag linked to a range of malaria antigens and cancer peptides. Spy-VLPs efficiently induced antibody responses after only a single immunization and without adjuvant. Plug-and-display VLP decoration has potential to accelerate vaccine development against a range of human and veterinary diseases.

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O9-5

Synthesis and characterization of new recombinant Elastin-Like Recombinamers fused to scFv for the formation of targeted polyplex

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According to the WHO, over 14 million new cases and 8.2 million cancer-related deaths are diagnosed every year. This has motivated researchers to develop innovative anti-cancer treatments, and Elastin-like recombinamers (ELRs) are an emerging tool in this field. ELRs are recombinant polymers inspired to natural elastin whose properties make them advanced biomaterials for several innovative biomedical applications. Their production by biotechnological procedures allow the total control over their chemical and physical features such as surface charge, polydispersity, self-assembly and biocompatibility. A single-chain variable fragment (scFv) is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins. The combination of both allows us to develop novel therapies that they can be applied in targeted anti-cancer drug administration to enhance treatment possibilities and avoid diffusion of the drug throughout the human body, thereby minimizing undesirable effects in healthy tissues.

scFv-ELR synthesis was done by molecular biology recombinant DNA techniques and expression in *E. coli* the purification was carried out by Ni-NTA affinity chromatography. Characterization of the obtained material included MALDI-TOF mass spectrometry, SDS-PAGE, western blot detection and densitometric analysis was performed with the objective of determining the degradation percentage of the biomaterial.

The ELR was characterized by sequencing, SDS-PAGE and western blot. The resulted ELR has less than 30% degradation, which allows its use in future studies.

This work describes the design, synthesis and purification of a novel ELRs with a target recognition. To conclude, this study opens the door to the use of ELRs to design targeted devices that in combination with a therapeutic pDNA.

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Symposium 10: Molecular Farming

O10-1

Re-engineering the tropane alkaloid biosynthesis pathway in potato

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Tropane alkaloids are a class of nitrogenous compound which have a significant role in the treatment of a myriad of medical conditions. Many members of the Solanaceae produce these high value tropane alkaloids, such as *Duboisia*, the commercial source. Potato, however, does not produce tropane alkaloids, although high levels of compounds which share precursors of tropane alkaloids do accumulate in potato tissues (nortropane alkaloids). Yields of tropane alkaloids from *Duboisia* are variable because of poorly understood genotype by environment interactions. The aim of this project is to investigate whether the range of alkaloids synthesised in potato can be extended by utilising biotechnological approaches to re-engineer the associated biochemical pathways, overcoming lesion points within potato. Transgenic lines have been developed to test whether metabolic flux can be diverted away from nortropane alkaloids towards the high value tropane alkaloid precursors. Transgenic lines which are over-expressing tropinone reductase I, have shown an accumulation of the tropane alkaloid precursor tropine, after application of exogenous tropinone. Similarly, down-regulating tropinone reductase II lines have shown a decrease in the accumulation of the nortropane alkaloid precursor pseudotropine; however, there was no statistically significant increase in the accumulation of tropine. Other regions of the biosynthetic pathway are under investigation through recombinant protein expression assays to generate a more in-depth understanding of this pathway within potato.

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O10-2

Degradation of anti-HIV antibodies 2F5 and PG9 by proteases of *Nicotiana benthamiana*

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The tobacco-related species *Nicotiana benthamiana* has emerged as a promising host for the manufacturing of protein therapeutics, but product yield and quality frequently suffer from limited proteolysis. When the human anti-HIV mAbs 2F5 and PG9 are expressed in the leaves of *N. benthamiana*, the heavy chains of both antibodies undergo partial proteolysis within their CDR H3 loops leading to the formation of discrete 40-kDa degradation products. Here, we provide evidence that these *in planta* cleavage events involve the action of serine proteases present in the leaf apoplast. *In vitro* processing of 2F5 by leaf apoplastic fluid was curtailed by the general serine hydrolase inhibitor FP-biotin, whereas inhibition of PG9 cleavage required the additional presence of other serine protease inactivators. FP-biotin was found to label distinct sets of apoplastic proteins sized 60–75 kDa. Mass spectrometric analysis of the polypeptides labelled with FP-biotin resulted in the identification of various subtilisin-like serine proteases. Future studies will address whether these enzymes are indeed responsible for the apoplastic cleavage of antibodies and other recombinant proteins produced in *N. benthamiana*.

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O10-3

Molecular farming in barley: production of human antimicrobial peptide LL-37

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The antimicrobial peptide LL-37 is an important native compound of the human non-specific immune system. Broad-spectrum activity of the peptide LL-37 against pathogens creates a premise for its potential application in the pharmaceutical industry. However, it is necessary to find a suitable platform for low-cost production of the peptide in relatively high amount. For this purpose, a novel platform for the production of antimicrobial peptide LL-37 in barley was developed, in which targeting to the grain endosperm allowed long lasting storage of the recombinant peptide in a biologically active form. Various constructs for barley transformation that contained different fusion epitopes driving an accumulation in specific cellular compartment and facilitating subsequent purification of the chimeric peptides were assessed. Corresponding gene expression was placed under the control of a maize ubiquitin promoter or barley endosperm-specific B1 hordein promoter. The transgenes were successfully integrated in the barley genome and also stably inherited in the next generations without any impact on plant phenotype. The peptide LL-37, which expression was driven by the B1 hordein promoter, was accumulated in quite high levels (0.55 mg/kg of grain) and exhibited antimicrobial activity.

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O10-4

Antimicrobial capacity of two optimized Mediterranean plant extracts against clinical isolated strains of *Staphylococcus aureus*

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Background: Multi-drug resistant *Staphylococcus aureus* is currently a health issue worldwide. Natural antimicrobial compounds arise as alternative or complementary treatments against nosocomial staphylococcal infections. Natural formulations containing several different molecules such as polyphenols have the ability to act against different bacterial molecular targets, avoiding common antibiotic resistance mechanisms.

Materials and methods: Two optimized herbal extracts (denominated CS and GP) from Mediterranean plants were selected from a previous screening of a big pull of natural extracts and pure compounds. The antimicrobial assays were performed *in vitro* using the broth microdillution method in p96 plates. Extracts were tested against 100 *S. aureus* clinical isolates: 50 methicillin-resistant (MRSA) and 50 methicillin-sensitive (MSSA) from the General University Hospital from Alicante. All the clinical isolates were characterized for resistance against fifteen different traditional antibiotics and tested for presence of the penicillin-binding protein 2a (PBP2a).

Results: Extracts showed mean MIC_{50} values between 50 and 80 μg/mL against both MRSA and MSSA. Interestingly, when comparing resistant and non-resistant bacteria for a given antibiotic, the MIC_{50} values were more dispersed and higher in most cases for the resistant ones. In general, CS extract worked best against antibiotic-resistant bacteria and GP extract was especially effective against antibiotic-sensitive bacteria.

Conclusions: The two extracts assayed have shown significant antibacterial activity against clinical isolates of *S. aureus* and are proposed for further synergy studies with traditional antibiotics against staphylococcal infections. These natural extracts may have potential to overcome bacterial multi-drug resistance mechanisms with no toxicity for potential patients.

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O10-5

Mycelium of *Lentinula edodes* enriched with selenium and study on release of this bioelement into artificial digestive juices

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Lentinula edodes is a culinary-medicinal mushroom species. This species is a source of lentinan, the best-known mushroom polysaccharide registered in a cancer therapy. Selol 5% (polish invention) is also a preparation used in a cancer therapy. Its uniqueness depends on the fact that it contains selenium at +4 oxidation level (in the form of selenitetriglycerides), which is safer for humans compared to the sodium selenate(IV).

The aim of the work was to combine the natural immunostimulating activity of the mushroom and the effect caused by organic selenium compounds. This objective was achieved by evaluating selenium accumulation in the *Lentinula edodes* mycelium enriched with Selol in two different selenium concentrations (25 or 50 mg/L medium). In order to confirm the possibility of action the enriched mycelium, the process of release selenium into artificial digestive juices was also conducted.

It was observed that the addition of selenium had a beneficial effect on increasing the biomass growth. In the control cultures, selenium was determined in amount 0.79 mg/100 g dry weight. However, after supplementing the culture media with selenium(IV), 192.65 mg/100 g dry weight (for the lower selenium addition) and 532.31 mg/100 g dry weight (for the higher selenium addition) were determined. Additionally, it has been proved that selenium is released efficiently into artificial digestive juices.

In conclusion, combination of the therapeutic properties of *Lentinula edodes* and selenium(IV) may potentially lead to obtaining a preparation strengthening anti-cancer activity and supplementing selenium deficiency in living organisms.

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O10-6

Effects of multi-strain lactobacilli supplementation on growth performance and immunostimulation in weaned pigs

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Many factors cause social and environmental stresses during the weaning period of pigs which leads to performance decreases and mortality increases. The aim of this study was to evaluate the effect of lactobacilli probiotics isolated from pig feces on growth performance and immunostimulation in weaned pigs. A total of 60 weaning pigs were divided into 5 groups (12 piglets per group) as follows: group 1 (control group) provided basal feed and water (B), foot and mouth disease vaccine (FMDv) and no probiotic, group 2 provided B, no FMDv and no probiotic, group 3 provided B, FMDv and *Lactobacillus plantarum* L21 (concentration at 10^9 CFU/ml), group 4 provided B, FMDv and the combination of 10^9 CFU/ml of *L. plantarum* (L21 and L80) and *L. paraplantarum* L103 and group 5 provided B, FMDv and commercial probiotic strains. The experiment started from day 21 to day 42. The results showed that supplementation of multi-strain lactobacilli (group 4) was the highest growth performance and enough for stimulating the humoral immunity to FMDv compared to the control group. Multi-strain probiotics in this study could be used together with the vaccination program in weaned pigs.

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Symposium 11: Industrial Biotransformation

O11-S

Computer-aided engineering of enzymes for *in vitro* and *in vivo* production of novel precursors

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Development of enzyme-based synthetic processes is often hampered by the lack of natural enzymes with requisite properties or specificities. With the potential offered nowadays by computer-aided molecular design and enzyme engineering techniques, we have seen in recent years numerous examples of successful enzyme designs that enabled tremendous improvements of catalytic properties for various applications, including catalysis of novel synthetic reactions. Nonetheless, progress in this field, in particular with computational techniques, is still required in order to fasten enzyme design and accelerate the generation of efficient biocatalysts.

This lecture will report and discuss recent developments and specific research projects of our laboratory. Special emphasis will be placed on the contribution of computational methods in our strategies.

Several areas will be covered: (i) development of computational methods for multi-scale molecular modelling and design inspired from artificial intelligence field [1]; (ii) computer-aided engineering of carbohydrate-active enzymes to conceive catalysts acting on non-natural substrates, to enter programmed chemo-enzymatic cascades, and ultimately produce antigenic oligosaccharide precursors [2]; (iii) structure-based engineering of enzymes to conceive an artificial metabolic pathway dedicated to in vivo production of non-natural methionine precursor [3].

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O11-1

A novel energy-efficient version of the Calvin cycle

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The use of organic carbon in growth media is one of the biggest costs in industrial fermentation. Autotrophic microorganisms which generate biomass by fixing CO_2 using the Calvin–Benson–Bassham (Calvin) cycle offer a more cost-effective alternative. However, the Calvin cycle has low energetic efficiency, consuming three ATPs and two NADPHs per CO_2 fixed. Here, we present data in support of a more energy-efficient version of the Calvin cycle hypothesized in chemoautotrophic gammaproteobacterial symbionts of marine invertebrates. These symbionts, which are some of the most prolific primary producers in the ocean, lack the gene for fructose 1,6-bisphosphatase (FBPase), an enzyme which catalyzes two essential reactions in the bacterial Calvin cycle. Using transcriptomics and biochemical enzyme characterizations in chemoautotrophic symbionts from a bivalve, *Solemya velum*, and molecular genetics in a closely-related free-living bacterium, *Allochromatium vinosum*, we demonstrated that a glycolytic pyrophosphate-dependent phosphofructokinase (PPi-PFK), acting in reverse, has the capacity to perform the function of the missing FBPase in the Calvin cycle. The shift from FBPase to PPi-PFK in *A. vinosum* was associated with an increase in thermodynamic efficiency at no detriment to the rate of CO_2 fixation. In sulfide oxidizing symbiotic bacteria, PPi produced by PPi-PFK in the Calvin cycle may be consumed by ATP sulfurylase (ATPS), the final enzyme in their sulfide oxidation pathway. ATPS can synthesize two ATPs per two PPis generated in the alternate Calvin cycle, lowering the "cost" of CO_2 fixation. Adaption of this pathway in bioprocess

engineering could offer a cost-effective alternative to traditional resource-intensive industrial fermentation.

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O11-2

Laccase: old enzyme with new applications

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Laccase (EC 1.10.3.2) is one of the earliest studied enzyme groups, while many innovative applications have been reported recently [1]. In nature, laccases oxidize a multitude of different substrates and are involved in many processes such as plant defense mechanisms, fungal pathogenesis, lignin degradation and polymerization [2]. We have exploited the latter ability to exploit lignosulfonates as an under-utilized by-product from the pulp and paper industry to replace fossil-based binders in conventional paper coating formulations [3]. For enzymatic polymerization of lignosulfonates, laccases such as from *Trametes hirsuta* were studied. Moreover, a new putative laccase from *Aspergillus flavus* (AfLacc1) was successfully engineered for improved activity on lignosulfonate. Therefore, a hydrophobic component, namely the substrate-binding domain of polyhydroxyalkanoate depolymerase of *Alcaligenes faecalis* was attached to AfLacc1. In addition, reaction engineering was conducted to avoid the use of expensive and/or toxic mediators. Indeed, continuous supply of oxygen was sufficient for polymerization of lignosulfonate, making mediators superfluous [4]. Laccase might be an "old" enzyme, but its potential is still not exhausted.

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O11-3

Intracellular metabolite profiling of *Streptomyces coelicolor* A3(2) in nutrient stressed batch fermentation for antibiotic production

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One task of the Centre for Digital Life Norway project INBioPharm is to develop optimized Streptomyces Superhost strains for heterologous production of new bioactive compounds by using Systems and Synthetic Biology approaches. One important experimental input to this task will be the generation of high-resolution quantitative metabolite profiles of the Streptomyces host production strains. In this study, wild type strain M145 of Streptomyces coelicolor A3(2), its mutant strain M1146 with the deleted biosynthetic gene clusters producing actinorhodin, undecylprodigiosin, CPK and CDA and M1152 having introduced point mutation into rpoB, were used for complete profiling of the intracellular primary metabolite pools.

Intracellular metabolite pools were quantified using several MS/MS based methods; i.e. two UPLC-MS/MS methods for amino acids and organic acids, a capIC-MS/MS method for nucleotides, sugar phosphate and other phosphometabolites. Fermentation data were used for mass balance, growth and production kinetics analysis. SSBM medium coupled with either L-glutamate or phosphate limitation triggered antibiotics production. Contrary to phosphate limitation, nitrogen (L-glutamate) limitation triggered the stringent stress response as observed by a sharp decline in the CO2 production and biomass concentration. The consumption of L-glutamate in broth reflected in the depletion of intracellular L-glutamate and the corresponding decrease in intracellular glutamine and α-ketoglutarate, an entry point of L-glutamate into metabolic pathway. 6-Phosphogluconic acid, first metabolite of pentose phosphate pathway (PPP) was found to rise with biomass generation showing increased PPP activity during growth phase. A greater activity of PPP ensures a continued supply of NADPH and precursor metabolites for highly dividing cells. The energy charge ratio was higher during mid-exponential and antibiotic production phase in comparison of stationary phase.

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O11-4

Ensuring sustained genetic stability and productivity during continuous culture of *E. coli* for the bio-production of citramalate

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Bio-production of platform chemicals by microorganisms from renewable feedstocks would reduce societal dependence on petrochemicals. However, current processes are restricted by the availability of cost-effective manufacturing processes. Although fed-batch fermentation processes are cost-effective for high value chemicals, they are less attractive for commodity chemicals, due to limitations of reactor size and process downtime. Continuous fermentation provides a potential solution, however poor

genetic stability of metabolically engineered production strains presents a barrier to implementation. The specific problem is that non-productive mutants appear in the culture and outcompete productive cells, due to their more efficient conversion of substrate to biomass. Our objective is to provide solutions to this problem, by developing genetically stable strains and demonstrating their use in continuous bioprocesses for chemicals production.

We have developed an engineered *Escherichia coli* strain which produces citramalate, a non-toxic precursor to methacrylic acid. To enable continuous bio-production in the absence of an inducer, we screened the Anderson promoter series to obtain optimised constitutive expression of the engineered pathway, and then tested strain stability in lab scale continuous culture. The plasmid was lost after 10 generations in glucose-limited cultures and 30–40 generations in phosphate limited cultures, resulting in loss of citramalate production. Therefore, we have developed and tested a number of synthetic biology solutions to ensure that expression cassettes are stably expressed during extended continuous culture, and also allow high productivity formation of citramalate. We are now applying these approaches to systems for production of a broader range of chemical products.

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O11-5

Antihypertensive activity of sesame residue-enriched soy milk fermented by lactic acid bacteria

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Angiotensin converting enzyme (ACE) is a key enzyme in the renin angiotensin system (RAS) responsible for conversion of angiotensin (Ang) I into Ang II, a vasoconstrictor leading to elevated blood pressure. ACE inhibitory peptides derived from food proteins have shown potential in the prevention and management of hypertension. In this study, Sesame residue-enriched soy milk media containing soy milk powder (8%), with added sesame residue (2%) were fermented by lactic acid bacteria and tested for bacterial growth and ACE-inhibitory activity. Then, sesame residue-enriched soy milk media was scaled up to 100 L fermentation and further prepared as lyophilized powder. The antihypertensive activity in spontaneously hypertensive rats (SHR) was investigated with or without lyophilized powder (sesame residue-enriched soy milk fermented by lactic acid bacteria). Oral administration of lyophilized powder at doses of 1.03 g/kg of body and after 6 weeks, the systolic pressure of the control group (without lyophilized powder) was 208.9 ± 2.1 mmHg, while the experimental group (with lyophilized powder) decreased to 186.2 ± 3.5 mmHg. The beneficial in vivo effects may be shown that sesame residue-enriched soybean milk media fermented by lactic acid bacteria have blood pressure regulatory effect.

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O11-6

Fungal biotransformation of 5-(hydroxymethyl)furfural into 2,5-di(hydroxymethyl)furan

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2,5-Di(hydroxymethyl)furan (DHMF) is considered a biobased diol useful for the preparation of biopolymers [1]. It can be obtained from 5-(hydroxymethyl)furfural (HMF) prepared from cellulose and hemicellulose by hydrolysis, isomerization and dehydration processes [2]. In this work, we report the biocatalytic reduction capability of fungi strains belonging to *Fusarium* (*F. striatum*, *F. sporotrichioides*, *F. tricinctum*, *F. poae*, *F. chlamydosporum*, *F. sambucinum* and *F. culmorum*), *Aspergillus* and *Penicillium* genus to produce DHMF from HMF. Microorganisms were grown in malt extract broth adjusted at pH 7 for 72 h before adding HMF. Although many of them were able to biotransform the substrate, different conversions were observed with an important effect of the HMF concentration. For instance, excellent substrate conversions (100%) and high DHMF yields (approximately 95%) were obtained with *F. striatum* with HMF concentrations up to 50 mM after 24 h. In addition, production of 5-methylfurfural was observed with low yields (approximately 5%). Biotransformation was also performed using the filtered culture broth, which could simplify the recovery of the products or the purification of the enzyme/s that catalyse the reduction of the HMF.

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Symposium 12: Tissue Engineering

O12-1

A hybrid thermosensitive hydrogel formulation for extended release of tacrolimus for vascularized composite allotransplantation

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A mixed hydrogel formulation consists of poloximer-poly(alanine)-poly(lysine) and pluronic F-127 was developed and tested for long-term delivery of anti-rejection drug tacrolimus. The composition, concentration and drug content were optimized for zero-order release over more than one month period in s.c. depot near the allotransplantation sites. The inclusion of Pluronic F-127 in this mixed hydrogel system was necessary to maintain a suitable drug release rate for successful anti-rejection outcome. *In vitro* and *in vivo* release rates as well as transplantation results will be presented.

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O12-2

Effects of a micro-derived antioxidant on semen parameters of males with asthenozoospermia

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Objective: To test effects of a micro-derived antioxidant, KB-120, on semen parameters of males with asthenospermia.

Method: Diagnosed according to WHO fourth edition semen analysis guideline, 40 males with asthenozoospermia were recruited, and given 1 g of oral antioxidant daily for three month. Semen parameters were examined at the beginning and every one month of the treatment.

Results: With one-month treatment, sperm concentration ($p < 0.01$), ratio of progressive motility (PM) sperms ($p < 0.01$), and ratio of rapidly progressive motile (RP) sperms ($p < 0.001$) significantly increased. At the second month, ratios of PM and RP sperms were higher than they were one month ago ($p < 0.05$). Compared to those at the second month, the semen parameters were not statistically changed at the end of treatment. And, during the treatment, there were 8 natural pregnancies (20.0%).

Conclusion: By significantly increasing the ratio of RP sperms, treatment of KB-120 was capable to improve the fertilizing capacity of males with asthenozoospermia.

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O12-3

Genistein-mediated correction of differential defects in the cytoskeleton in cellular models of various neurodegenerative diseases

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Sanfilippo disease (mucopolysaccharidosis type III, MPS III) and Huntington's disease (HD) are neurodegenerative disorders caused by accumulation of undegraded heparan sulfate and mutant huntingtin, respectively. Changes in the cytoskeleton were previously suggested as secondary cellular effects in both diseases. We asked what are the specific changes in the cytoskeleton in MPS IIIB and HD cellular models, and whether they can be corrected by genistein, a compound previously demonstrated to be capable of reducing levels of both heparan sulfate mutant huntingtin, in a potential therapeutic procedure called gene expression-targeted isoflavone therapy (GET IT). Cellular models of MPS IIIB (patient-derived fibroblasts) and HD (HEK-293 cells transfected with plasmids encoding mutant huntingtin) were used, with appropriate controls. Microfilaments and microtubules were visualized by fluorescence microscopy. Levels of beta-actin and alpha-tubulin were estimated by Western-blotting. No changes have been observed in the microfilaments of MPS IIIB and HD cells. However, severe deformations of microtubules were visible in both cellular models of the diseases. Higher amount of alpha-tubulin and denser cytoskeleton were observed in MPS IIIB cells while decreased levels of alpha-tubulin and underdevelopment of microtubules occurred in HD cells. Intriguingly, genistein successfully corrected levels of alpha-tubulin and the structure of the cytoskeleton in both MPS IIIB and HD cellular models. We suggest that despite different kinds of defects in the cytoskeleton in MPS IIIB and HD cells, genistein corrects all of them through alleviation of the primary causes of

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these diseases, i.e. accumulation of heparan sulfate and mutant huntingtin, respectively.

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O12-4

Generation of macroporosity in 3D scaffold using a combined approach of high-speed stirring and freeze-drying method: potentials in bone tissue engineering application

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Introduction: In bone tissue engineering, among all the physical parameters, pore size and porosity play an important role in determining successful tissue regeneration. Interconnected pores ranging from 100 μm to 1000 μm are essential for cellular and vascular invasion. In the present work, an approach of combined high-speed stirring and freeze-drying method was attempted to fabricate macroporous scaffolds. The rationale behind the idea is that proteins are surface active molecules and ultrasonic agitation increased its surface activity with foam stability and bubble uniformity. Gelatin and CMC biopolymers were chosen as base biomaterials while nano-hydroxyapatite was added as an inorganic component of the biomaterial.

Methods: The macroporous scaffold (SGC) was characterized by their physicochemical properties using SEM, FTIR, XRD, UTM, and CT-SCAN. Mesenchymal stem cells microtissue (MSC-MT) viability, proliferation differentiation towards osteoblasts and mineralization rate was evaluated on SGC scaffold as a function of time. The MSC differentiated osteogenic gene expression profile of was assessed using RT-PCR.

Results: The SGC scaffold showed augmentation in properties like swelling, porosity, pore size distribution, biodegradability, osteoconductivity, and osteoinductivity than conventional freeze-dried scaffolds. Live–dead staining and Alamar blue assay showed an increased adhesion and proliferation rate of MSC-MT on SGC scaffold. Additionally, expression of differentiated osteogenic genes was also observed in the macroporous scaffold.

Conclusion: Our study demonstrated that SGC scaffold enhanced the cellular viability, proliferation, and functionality of differentiated MSC-MT. This probably could be due to macroporosity which enhances the diffusion of nutrients and waste products into and out of the scaffold. Additionally, the high speed stirring evenly distributed nHAp throughout the scaffold, which significantly increases the osteoconductivity and osteoinductivity of the scaffold.

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O12-5

iPSC-derived neurospheroids recapitulate development and pathological signatures of human brain microenvironment

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Brain microenvironment plays important roles in neurodevelopment and pathology. Neural cell culture typically relies on heterologous matrices that poorly resemble brain ECM or reflect its pathological features. We have shown that perfusion bioreactor-based 3D differentiation of iPSC-derived human neural stem cells (hiPSC-NSC) sustains the concomitant differentiation of the three neural cell lineages (neurospheroids). We hypothesized that if neurospheroid strategy also allow deposition of native neural ECM, it would be possible to (i) mimic cellular and microenvironment remodeling occurring during neural differentiation and (ii) recapitulate pathological features of diseases in which alterations in cell–cell interactions and ECM are relevant. Quantitative transcriptome (NGS) and proteome (SWATH-MS) analysis showed that neurogenic developmental pathways were recapitulated in our system, with significant changes in cell membrane and ECM composition, diverging from 2D differentiation. We observed a significant enrichment in structural proteoglycans typical of brain ECM, a downregulation of basement membrane constituents and higher expression of synaptic and ion transport machinery. Neurospheroids were generated using hiPSC-NSC derived from Mucopolysaccharidosis type VII (MPS VII) patient. MPS VII is a neuronopathic lysosomal storage disease caused by deficient β-glucuronidase (β-gluc) activity, leading to glycosaminoglycan (GAGs) accumulation in the brain. The main MPS VII molecular hallmarks were recapitulated, namely GAGs accumulation. MPS VII neurospheroids showed reduced neuronal activity and disturbance in network functionality, with alterations in connectivity and synchronization. These data provide insight into the interplay between reduced β-gluc activity, GAGs accumulation, alterations in the neural network, and its impact on MPS VII-associated cognitive defects.

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O12-7

Preparation of nitric oxide-releasing photo-crosslinked electro-spun chitosan nanofibrous scaffolds for bone tissue engineering

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In this work, sodium nitroprusside-releasing chitosan-based (CS/SNP) nanofibers were fabricated via electrospinning. Pre-pared CS/SNP nanofibers were capable of sustainably releasing 37 μg SNP/mg for up to 7 days. SNP is known to release nitric oxide (NO).

To improve nanofiber stability and mechanical properties, one-step photo-crosslinking of blended CS/SNP nanofibers was carried out by addition of tetraethylene glycol diacrylate (TTEGDA) and 2,2-dimethoxy-2-phenylacetophenone (DMPA), and incorporation of UV irradiation into the electrospinning process. Application of photo-crosslinking was found to significantly improve nanofiber stability in aqueous environments. SEM images revealed that the porous nanofibrous structure could be maintained up to 24 h. Biocompatibility of CS/SNP nanofibers towards mouse osteoblasts was also significantly improved.

Addition of SNP into the nanofibrous scaffolds were found to improve their biocompatibility to osteoblasts and gingival fibroblasts (GF). Cell viability of 7F2 mouse osteoblasts and human GF cells were affected by SNP content in a dose- and time-dependent manner. MTT assays revealed that 7F2 cell viability increased with increasing SNP content, whereas GF cell viability peaked in CS/20% SNP nanofibers. Fluorescence microscope images also revealed that CS/SNP nanofibers improved cell attachment, spreading and proliferation. Osteogenic differentiation and mineralization were also enhanced by the nanofibers, as evidenced by elevated expressions of osteogenic differentiation markers including alkaline phosphatase (ALP), osteopontin (OPN) and calcium. Photo-crosslinked electrospun CS/SNP nanofibers are thus shown to have excellent potential as bone tissue engineering scaffolds.

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Symposium 13: Biopharmaceutical Processing

O13-S

Full-length human CCBE1 production and purification: Leveraging bioprocess development for high quality glycosylation attributes and functionality

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Collagen and calcium-binding EGF domain-1 (CCBE1) is a secreted protein critical for lymphatic and cardiac vascular development and regeneration. However, the lack of enough amount of recombinant full-length CCBE1 (rCCBE1) has limiting its potential in tissue regeneration as a therapeutic factor. The main goal of this work was to implement a robust bioprocess to efficiently produce high amount of glycosylated and functional full-length rCCBE1. Different bioprocess strategies were combined with proteomic tools for process and product (CCBE1) characterization, evaluating the impact of process parameters on cell performance, rCCBE1 production and quality.

We have shown that rCCBE1 volumetric yield was positively correlated with higher cell density at transfection (HDT), and under these conditions the secreted protein presented a mature glycosylated profile (complex N-glycans). Mild hypothermia was also applied to HDT condition that resulted in enhanced cell viability; however an enrichment of immature rCCBE1 variants was detected. Mass spectrometry-based tools allowed the identification of rCCBE1 peptides confirming protein identity in the affinity chromatography purified product. rCCBE1 functionality attributes were confirmed, namely its angiogenesis potential, by evaluating vessel formation in an in vitro cell-based assay.

Herein, we report a step forward in the production and characterization of human glycosylated full-length rCCBE1, amenable for in vitro and in vivo studies to further explore its regenerative therapeutic potential for cardiovascular repair.

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O13-1

Identification of novel promoters and genetic control elements derived from Chinese Hamster Ovary cells

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For recombinant protein production in mammalian cell lines a high rate of gene expression is desired, therefore, strong viral promoters are commonly used. However, these have several drawbacks in that they override cellular regulation and responses, are not integrated into the cellular network and thus induce stress and potentially epigenetic silencing. Endogenous promoters might have the advantage of better response to cellular signaling networks and thus cause a lower level of stress by uncontrolled overexpression of the transgene. Here we describe the identification of endogenous promoters and their regulatory elements from Chinese hamster ovary cells based on histone mark and chromatin state data previously generated [1]. A promoter candidate list was generated based on the top expression of both coding genes and lncRNAs and tested for normalized promoter strength in comparison to the Cytomegalovirus promoter with enhancer using a dual luciferase kit. Successive truncation of the promoters at the 5′ and 3′-site of the initial DNA fragments further enhanced promoter functionality in some of the cases and may lead to a better understanding of promoter architecture in the future. Performance in stable recombinant cell lines is currently being tested at a targeted integration site for better comparability. Besides promoters, the chromatin state analysis [1] also enables identification of other regulatory elements, such as enhancers, for improved expression level. Different enhancers were cloned upstream of endogenous and viral promoters and observed to further enhance promoter activity up to 3 fold. However, the specific combination had major impact on enhancer effect.

Reference

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O13-2

Recombinant human interferon alpha-2β: Cloning, expression and PAT-process analytical technology enabled production in glycoengineered *Pichia pastoris*

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USFDA approved recombinant IFN α-2β drugs are currently available in the market for the treatment of chronic hepatitis B and C and specific cancer ailments. However, the clinical outcome was not satisfactory because of short plasma half-life, rapid clearance and other side effects. To circumvent this problem, glycoengineering strategy was successfully employed to construct IFN α-2β with potential N-glycosylation site. Further to overcome the problem of hypermannosylation in the wild type strains of *Pichia pastoris*, glycoengineered strain (SuperMan5) was employed to express IFN α-2β with human type glycans. Glycan analysis elucidated that the synthesized IFN α-2β was N-glycosylated, and Man5GlcNAc2 was the major N-glycan attached to it. The glycosylated IFN α-2β was biologically active with no loss in the antiviral activity against HCV and HEV systems as compared to standard. Pharmacokinetics studies showed 1.3 fold increase in plasma half life for glycosylated IFN α-2β compared with non-glycosylated IFN α-2β in Wistar rats. Dielectric spectroscopy and respirometry were successfully employed as PAT tools for real-time monitoring of IFN α2β fermentation process. Real-time capacitance data revealed strong influence of specific growth rate on IFN α2β production. An estimator for specific growth rate (μ) was developed based on capacitance measurements and a feed-back control strategy was deployed for real-time specific growth rate during the induction. Significant enhancement of IFN α2β titre (1484 mg/L) was attributed at an optimal set point ($\mu = 0.04\ h^{-1}$), which is the highest titre reported in literature till date.

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O13-3

Developing of an efficient membrane based downstream processing for oncolytic measles viruses

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Since Measles viruses have shown its oncolytic effect first in the early 70s (Bluming and Ziegler, 1971), Measles viruses became highly interesting for the application in cancer treatment. However, one therapeutic dose for cancer treatment must contain at least 10^{11} infectious virus particles ($TCID_{50}$, vaccination: ~10^3 $TCID_{50}$) (Russell et al., 2014). This shows the importance of a high titer virus production and its subsequent downstream processing (DSP). In DSP, the full recovery of oncolytic Measles virus (OMV) infectivity is targeted. Besides this, the depletion of impurities, such as host cell proteins (HCP) and host cell DNA (hcDNA), to appropriate limits set by regulatory authorities have to be reached. This highlights the need of an efficient downstream processing.

In our institute, we established a high titer production process, achieving OMV titers of 10^{11} $TCID_{50}$ mL^{-1} (Grein et al., 2017). Now, we are focused on the downstream processing of OMV. For this purpose, we characterized the OMV regarding important process parameters. Additionally, agglomeration and the influence of shear stress were investigated. For the purification of OMV, a first clarification step was conducted. This was followed by a tangential flow filtration (TFF). Therefore, polyether sulfone flat sheet membranes were investigated in concentration mode. We were able to recover the infectious virus and lowered the impurities by ~70% for hcDNA and ~80% for protein content. A further applied discontinuous diafiltration depleted the impurities by ~95% in total. These results showed that TFF is an appropriate tool for the purification and formulation of OMV.

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O13-4

Characterization of antibody drug conjugates by an innovative on-line four-dimensional HICxSEC-IM-MS methodology

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Antibody drug conjugates (ADC) are extremely challenging to characterize because of their inherent structural heterogeneity. A combination of orthogonal analytical techniques is generally required to investigate several critical quality attributes (CQAs). Two main techniques are used for this purpose in non-denaturing conditions, namely the hydrophobic interaction chromatography (HIC) and the native mass spectrometry (MS). However, both techniques can lead to misinterpretations or incomplete characterization when used as standalone and a direct hyphenation of HIC to MS is not possible, because of the presence of large amounts of salts in the mobile phase.

Here, we present an innovative multidimensional (4D) approach consisting of comprehensive two-dimensional chromatography, namely HIC in the first dimension and size exclusion chromatography (SEC) in the second dimension, coupled on-line to ion-mobility and mass spectrometry (IM-MS) for performing comprehensive and streamlined characterization of both native and forced degraded ADC samples. In this 4D configuration, the integrity of HIC separation was maintained; SEC was used for its size-based separation capabilities and exclusively applied as "fast-desalting" step, while a global IM-MS picture within a single run was also provided [1].

Altogether, our results demonstrate for the first time the ability to have a comprehensive analytical characterization of an ADC within a single run, affording: (i) simultaneous drug load profile and quantitative average DAR assessment (HIC); (ii) unambiguous identification of the number of drug conjugations through accurate intact mass measurement (native MS); and (iii) conformational homogeneity assessment of each drug load species (IM).

Reference

[1] Anal Chem 2018;90:1578–86.

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O13-5

Generation of novel bioactive "unnatural" natural products through biotransformation by the enzymatic fungal secretomes

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Biotransformation can be defined as the use of an intact whole organism or an isolated enzyme system to induce chemical modifications in organic compounds. Biotransformation has a number of advantages when compared to classical organic chemistry. The reactions can occur in mild conditions, near neutral pH, ambient temperatures, and atmospheric pressure, and protection of certain functional groups is often not necessary. For performing biotransformation of Natural Products (NPs), the protein secretome from fungi could be considered as a promising tool. Fungi are indeed of interest since they synthetized diverse and complementary extracellular proteins to permit host penetration. This group of secreted proteins released in the extracellular space is defined as the secretome and constitutes the pathogenicity factors in the host-pathogen interaction mechanisms. In the present work, the protein secretome of *Botrytis cinerea* was used to perform the biotransformation of some NPs. The reactions were optimised and monitored at the analytical scale. Metabolite profiling by UHPLC-HRMS are used to detected compounds with unusual molecular formulas. The reactions were scaled-up a series of unusual analogues were isolated and fully characterized by NMR and HRESIMS analyses. The biological properties of the compounds generated were evaluated using *in vitro* bioassays, for example against multi resistant's strains of *Staphylococcus aureus*. Some of the isolated compounds present a strong antimicrobial activities showing that an inactive molecule, can be modified into more active compounds after enzymatic biotransformation. Our results suggest that biotransformation by usage of crude fungal secretomes represents an efficient way to create chemodiversity with enhanced biological activity.

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O13-6

The challenges and opportunities of cascading enzymatic microreactors

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The pharmaceutical industry has an increased interest in the continuous production of high value or difficult to synthesize products. In chemical synthesis cascading reaction system have already been employed with significant success, allowing a quick change in reaction conditions and addition of new reactants as well as removal of undesired side products. Additionally, these systems can remove the need for isolating unstable intermediates, concomitantly increasing the yield of a synthetic pathway. Based on the success for chemical synthesis, the question arises how cascading systems could be beneficial to both chemo-enzymatic and biocatalytic synthesis. Structured microreactors are promising tools for the development of such processes and are frequently used to find novel routes for process intensification and improved process economics due to their unique characteristics.

In this contribution, we will present examples of cascaded microreactors giving special attention on how microreactors are combined and the challenges as well as opportunities that arise from such combinations. The enzymatic synthesis of 2-amino-1,3,4-butanetriol and chemo-enzymatic synthesis of 1-(3,4-Dimethyl-3-cyclohexen-1-yl)-1,3-dihydroxypropan-2-one, precursors of statins and 3,4-dimethylcyclohex-3-ene-2′-keto-1′,3′-propanediols, respectively, will be used to illustrate this concept. State of the art of online monitoring for enzymatic microreactor cascades will be presented and illustrated with the integration of optical pH sensors into our microreactors monitor reaction progress in real time. Finally, work-up and purification steps and their integration with microreactor cascades will be show cased with the chiral resolution of methylbenzylamine, highlighting the potential and the challenges of integrated cascades.

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Symposium 14: Improvement of Food and Feed

O14-S

Novel biopesticides. Functional peptides, from nature to the biotechnological product

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Losses due to pests account for near one third of the potential crop productivity, in spite of the actions taken for its control. In addition, plant food products' safety is currently an important issue in food production. Pesticides have greatly contributed to improve crop productivity and food security, but with non-target effects due to adverse impact into environment and human health. The rising of new emerging plant pests, require novel pesticides for the new scenario of sustainable agriculture, and biopesticides from living organisms offer an alternative or complement to conventional pesticides for plant disease and pest control. Functional peptides have been proposed as novel biopesticides. However, natural sources of functional peptides have the disadvantage that the active compounds are at low concentrations, and generally require complex extraction procedures or may be toxic. Synthetic peptides can be developed departing from natural models to optimize their properties by means of a structure-function approach. Peptide libraries are screened attending to the desired properties (antimicrobial, plant defence elicitation, insecticidal, nematicidal, etc.). In addition, the peptides are submitted to studies to discover its mechanism of action and to evaluate their toxicity. Leads are finally tested in proof-of-concept assays in plant hosts under greenhouse or field conditions.

One of the limitations for the use of functional peptides in agriculture is their sustainable production, and microbial or plant biofactories have been developed with several strategies for the heterologous expression of functional peptides. The prospects and limitations of functional peptides as novel biopesticides will be discussed.

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O14-1

Optimization of gamma-PGA biosynthesis supported by synthetic biology and metabolic engineering strategies

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Poly-γ-glutamate (γ-PGA) is a natural polymer made of glutamic acid residues, synthesized by the *pgs* operon of *Bacillus subtilis*. γ-PGA has a wide range of applications as food, cosmetics and pharmaceutical additive. However, to increase its industrial attractiveness, it is necessary to cut production costs utilizing cost-competitive feedstocks for fermentation. Raw glycerol is a low-cost by-product of biodiesel plants (it accounts for 10% of the final product) that can be used as feedstock. To achieve cost-competitive γ-PGA production from glycerol a multifaceted approach has been set up that includes:

(1) Characterization and optimization of *pgs* operon regulation: the strength of the *pgs* operon regulatory elements has been analysed both by a synthetic biology approach, exploiting the well-characterized expression operating unit (EOU) inserted in *amyE* [Guiziou et al., 2016], and by a classical *in-locus* transcriptional fusion. Results from the two settings will be compared. These data will now be used to finely tune *pgs* expression through an inducible promoter to optimize γ-PGA yield.
(2) Accumulation of γ-PGA precursors by metabolic engineering: a genome-scale metabolic model [Oh et al., 2007] was used to identify suitable targets for enhancing central carbon pathway flux toward γ-PGA synthesis. The first two *B. subtilis* strains, engineered according to this analysis, showed enhanced polymer production. Other target genes are under investigation.
(3) Enhancement of glycerol metabolism: *B. subtilis* tolerance to raw glycerol obtained from a biodiesel plant (from both vegetable and animal origin) was verified. Further investigations are underway to improve glycerol uptake and consumption.

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1871-6784/

O14-2

Nephroprotective roles of potent medicinal plants against acetaminophen (N-acetyl-P-aminophenol) induced kidney damage in mice

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Paracetamol (acetaminophen) is anti-inflammatory drug used as analgesics worldwide whose overdose could be damaging to kidneys in human and animals. Medicinal plants offer potential to protect kidneys against nephrotoxicity.

This study was conducted to evaluate Geranium wallichianum, Thymus serpyllum, Elaeagnus parvifolia and Viola canescens plants against paracetamol induced nephrotoxicity in albino mice. Mice were divided into six groups with three mice in each group. Group I was given no paracetamol and was termed as normal control. Group II was paracetamol control (PC). Group III was treated with PC and methanolic leaves extract of Geranium plant. Group IV was treated with PC and methanolic leaves extract of Elaeagnus plant. Group V was treated with PC and methanolic leaves extract of Thymus plant. Group VI was treated with PC and methanolic leaves extract of Viola plant. On fifteenth day, mice were sacrificed to draw their blood and kidneys for further examination. Biochemical results showed significant increase of Urea, Creatinine and Uric acid levels in the blood serum of mice of group II ($P < 0.001$). On the other hand, these levels were significantly reduced in groups III–VI. Histopathological examination of the renal sections from mice treated with only PC showed significant damages to the renal tubules and renal corpuscles, whereas renal sections from mice treated with PC and plants leaves extracts exhibited normal physiology. Therefore, it is concluded that crude methanolic extracts of all four treated plants have nephroprotective role against Paracetamol induced nephrotoxicity which could be due to their antioxidant properties.

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O14-4

Acidogenic fermentation of spent coffee grounds

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Spent coffee grounds (SCG), the waste that results from the brewing process, are produced in high amounts and contain many diverse organic compounds, including carbohydrates, fatty acids, and phenolics. SCG can be valorized in numerous ways, including the production of polyhydroxyalkanoates (PHA) by pure cultures. This process requires operation under extremely controlled conditions and sterility, which contributes to the increase in production costs. Mixed microbial cultures (MMC) can produce PHA using wastes or industrial by-products as substrates, allowing for their valorization. The use of wastes, together with the lower requirements of sterility and process control, could signify a decrease in PHA production costs. PHA production costs are considered one of the main drawbacks that prevent the increase of world market share of these polymers. The ability to store PHA provides microorganisms a competitive advantage for survival under transient conditions typical of wastewater treatment systems. In this way, MMC can continuously adapt to the operational conditions increasing the number of PHA-storing organisms with minimum requirements of sterility.

The objective of this work was the conversion of carbon compounds present in SCG to short chain organic acids (SCOA) since SCOA are the preferred substrates by MMC to produce PHA. Direct fermentation of SCG was carried out in a continuous stirred tank reactor (CSTR) where the operational conditions that enriched the microbial population in acidogenic organisms were evaluated. Several organic loading rates and hydraulic retention times were tested in order to achieve the highest amount of SCOA with the best composition for PHA production. The produced SCOA will be utilized for the selection of a PHA-storing MMC and in accumulation assays.

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O14-5

Pretreatment of coffee silverskin with ultrasound and mild alkaline solutions for enhancement of sugar yield

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The increasing global energy requirement is shifting the scientific attention from fossil to bio-based resources to produce energy and chemicals. Lignocellulose is the most abundant natural and renewable resource on Earth. A considerable amount of such material is generated as waste through agricultural practices mainly from various agro-industries. Agriculture food processing wastes (AFWs) are potential feedstock for biorefinery processes being economic and eco-friendly. Coffee silverskin (CSS) is an AFWs produced during the coffee beans roasting process. These vegetable residues are recalcitrant to enzymatic and microbial attacks, limiting their use for biorefinery applications. Pretreatment delignification methods are required to facilitate the enzymatic hydrolysis of AFWs aimed at the recovery of monomeric fermentable sugars from these residues. The aim of this study was to develop a pretreatment process by using ultrasound and mild alkaline solutions for the effective separation of lignin and cellulose to improve the sugar yield from CSS. The effects of sonication time, biomass loading, sodium hydroxide concentration and residence time in autoclave, were studied using Response Surface Methodology (RSM). A maximum reducing sugar yield of 0.6 $g_{sugar}/g_{total\ sugar\ in\ pretreated\ biomass}$ was obtained with 5 min sonication, 11% w/v biomass loading, 5% w/v NaOH and 75 min autoclave. Analysis of liquid after pretreatment revealed that fermentation inhibitors like furfural, HMF, ferulic and p-coumaric acid were absent or present in non-toxic concentrations for various *Clostridium* sp. Moreover, a phenolic content of 25.3 $mg_{GAE}/g_{raw\ CSS}$ was found. Changes of biomass structural properties after pretreatment were highlighted by SEM and XRD analysis.

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O14-6

Lab scale fermentation studies with marine bacteria to maximize the production of bioemulsifiers for cosmetic and food industries

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The behaviour of a novel marine bioemulsifier producing bacterial strain was studied under standard conditions in a modified Zobell marine medium. A statistical analysis based on a factorial design was adopted to determine the optimized culture conditions for enhancing bioemulsifier production. Medium composition and culture conditions were optimized by evaluating the effect and interaction of different factors (nitrogen sources, pH, temperature and salinity) on dry cell biomass and water-in-oil and oil-in-water emulsification capacities. The potential of the marine microorganism to utilize hydrophilic (glucose) and hydrophobic (rapeseed oil) carbon sources was also assessed. The resulting optimized media conditions increased the maximum growth rate value from 0.030 to 0.042 h^{-1} and enhanced the bioemulsifier production (1.54 ± 0.25 g/L) in two folds.

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O14-7

Phytate degrading probiotic bacteria as biocatalyst for commercial applications in food/feed industry

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Use of microbes as biocatalyst specifically lactic acid bacteria (LAB) belonging to group of probiotics are of immense importance as they have multifaceted health benefits, categorized into two aspects: nutritional and therapeutic. In present study we have studied functional attributes of lactic acid bacteria for potential commercial applications as probiotics in food/feed industries. The isolated LAB wereexamined for their ability to produce extracellular enzymes, i.e., amylase, protease, lipase, phytase andcellulase. None of the isolates showed extracellular protease and cellulase producing ability, however, only a few LAB isolates exhibited lipase and phytase producing ability. Almost all the isolates had amylase producing ability. An essential prerequisite for a potential probiotic is to have high (>90%) survival rate while transiting to harsh gastrointestinal conditions. Studies were carried out on survival rate of LAB in simulated gastric conditions i.e. low pH and high bile tolerance. A comparative study among Lactococcus and Lactobacillus strains, isolated from fermented food products have shown survival rate more than 90% in simulated gastric conditions forLactococcus strains K.J (a) and K. These two strains had an additional probiotic characteristics i.e. phytase producing ability. Phytaseshydrolyzephytates, ananti-nutritional properties of phytate could be minimized or omitted with the application of phytase/phytase-producing probiotics. Lactococcus strains K.J (a) and K exhibited substantial phytase-producing ability at 37 °C (0.555 U/ml and 0.583 U/ml respectively) in addition to other functional probiotic characteristics, auto-aggregation of the strains was high, and various degrees of co-aggregation was observed with indicator pathogenic strains.

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Symposium 15: Novozymes Integrated Bioprocessing Symposium part 1

O15-1

Cross-industry applications for leveraging integrated process analytical technology (PAT) and data analytics applications

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Despite the significant differences between the brewing and bioproduction industries, there are a surprising number of principles that hold true for both processes. As a result, meeting a biopharmaceutical scientist who proclaims a love of brewing is not unusual. Both brewing and bioproduction provide an opportunity to delve into a complex, specialized process to understand the science involved. In fact, there are combinations of data analytics methodologies and PAT applications in use in other industries today that can be brought into the bioproduction environment. These tools provide the scientific insight required for robust process understanding.

Taking advantage of integrated PAT and data analytics strategies that have been combined and successfully implemented in other industries is an opportunity to take product development to new levels. Using data-rich case study examples, this presentation will illustrate the cross-over potential for techniques applied successfully in the brewing and pharmaceutical industries, which can be of benefit to both. For example, tailoring the PAT options with the ability to rapidly analyze and derive insight using batch data from on-line process sensors and off-line analytical instruments can ensure the right data is collected and analyzed effectively.

Pharmaceutical companies continue to invest significant resources in the development of their pipelines, generating significant data assets in the process. These assets can be used by scientists to lead their teams toward more targeted experimentation, more efficient and rapid data investigation efforts. These elements can lead to improved cost and resource management – and most importantly an improvement in quality and production rates.

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O15-2

Production of antioxidants compounds for food and cosmetic application obtained by fungal liquid media cultures

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Identification of new natural molecules with biological activities is constantly required in many industrial fields. According to this, several microorganisms are studied due to their capacity to produce bioactive compounds with anti-microbial, anti-cancer and antioxidant power. Within this context, the filamentous fungus *Aspergillus tubingensis*, represents an innovative proposition of study since it produces high value metabolites as it is the case of Nafto-gamma-pyrones (NGP's) (Nielsen et al., 2009).

Therefore, the main objective of the present study was the optimization of NGP's production by A. tubingensis. For this purpose, fungal cultures were performed in CYB (Czapek Dox Broth) liquid media and a variation of different growing parameters (pH value, temperature, static and agitation conditions) were evaluated. Total quantification of the NGP's was performed by HPLC (High Performance Liquid Chromatography) and biological and physical properties were analyzed by TEAC (Trolox Equivalent Antioxidant Capacity) and colorimetric methods such as YAN (Yeast Assimilable Nitrogen), DNS (3,5-Dinitrosalicylic acid) and Nitrogen, Nitrate Colorimetric Brucine.

Results demonstrated that the highest amount of NGP's was produced in day 9 under static conditions at 28 °C, using a CYB media composition with 3 g/L of NaNO3 at pH 5. Results also demonstrated that agitation conditions favor the production of fungal biomass while static conditions favor fungal sporulation. In addition, a significant alteration of fungal morphology was observed depending on the culture conditions. In conclusion, we demonstrated that the present study could be considered as an effective method to increase NGP's production and represent a novel strategy to industrial processes.

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O15-3

Integrated ABE fermentation–adsorption process for enhanced butanol production by *Clostridium acetobutylicum*

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The biotechnological route to produce butanol via Acetone–Butanol–Ethanol fermentation still suffers of several disadvantages. On one hand, the low maximum butanol concentration due to the butanol toxicity to microorganisms and the low specific butanol productivity severely limit the industrial development of the butanol production. On the other hand, a low-cost and energy consuming technology to recover the butanol from the low-concentration broth is still a target of the research. The recovery and concentration of butanol by means of adsorption technique has several advantages in terms of biocompatibility and stability, as well as of the process economy. Therefore, the butanol recovery by adsorption has high potentiality for industrial application.

The aim of this contribution is to report recent results of a study carried out in Napoli as part of the HORIZON2020 project "Sustainable production of next generation biofuels from waste streams – Waste2Fuels" (GA – 654623).

Clostridium acetobutylicum was used for the continuous fermentation process. The conversion was carried out in 4 packed bed biofilm reactors (PBBRs) connected in series: the first reactor of the series was kept under acidogenesis, the successive reactors were kept under solventogenesis. A glucose bearing solution was continuously fed to the reactors.

The adsorption/desorption process was carried out in fixed-bed column for butanol–water and for synthetic fermentation broth model solutions. Amberlite XAD-7 was used as adsorbent material.

The integration of the PBBRs with an adsorption recovery unit was successfully developed and operated.

The butanol productivity and the final concentration of butanol were very competitive.

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O15-4

Ferulic acid synthesis in engineered *E. coli* is limited by methyl-group supply

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The phenylpropanoic acids coumaric acid, ferulic acid and sinapic acid are three precursors of lignin as well as a set of secondary metabolites of which some are predicted to have beneficial biological activity on human health. To identify potential biotechnological routs for their synthesis, we investigated the production of ferulic acid in *E. coli*. We designed an inducible polycistronic expression construct comprising four enzymes catalysing the immediate three step conversions from tyrosine to ferulic acid by desamination (TAL), aromatic hydroxylation (HpaBC) and methylation (OMT). Recombinant cells cultivated in minimal medium were pulse feeded with tyrosine and the concentration of intermediates and product were followed by quantitative HPLC measurements. In initial shake flask cultivations a set of homologous enzymes were assessed individually and the best candidates implemented in the synthetic cascade. Cells expressing all enzymes of the cascade transformed 2 mM tyrosine in 0.5 mM ferulic acid in a shake flask in 24 h, by concomitant accumulation of 0.3 mM caffeic acid. Addition of methionine and serine, precursors of S-adenosyl-methionine, improved the final ferulic acid concentration to 1 mM by improved methylation of caffeic acid. Theoretical flux analysis based on kinetic data of the first two enzymes of the cascade revealed a substantial downshift in the flux by a strong product inhibition of the first enzyme ($K_I = 11\ \mu M$) together with a high K_M of the second enzyme ($K_M \approx 1$ mM). Product inhibition is partially relieved in a ΔtyrR-background. In a controlled reactor environment with constant feeding of 1.5 mM/h tyrosine, the ΔtyrR-strain yielded 5.5 mM ferulic acid and 1 mM caffeic acid after 24 h.

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O15-5

Effect of water on microorganisms in solid state bioprocessing

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Water has a significant impact on microorganisms. It affects their viability as well as their productivity and growth rate. Traditionally, moisture content, water activity and other descriptions have been used to quantify water availability for microorganisms. However, none of the existing descriptions is totally sufficient, especially for microorganisms growing on solid substrates. For example, physical and chemical bindings of water molecules reduce water activity. But based on our experiments conducted on gels and superabsorbents, the reduction in water activity was not reflected in an equivalent reduction in bio-availability of water to microorganisms.

On the other hand, depending on their properties, different solid substrates have different water affinity and consequently different ability to absorb water from surrounding gas. Our experiments with Aspergillus Oryzae show absorbance of water into the solid phase enables fungi to effectively utilise water vapour. Therefore when describing water availability in a solid state bioprocessing system, it is necessary to take humidity into consideration.

We also tested water diffusion rates for different substrates and found that they vary with substrate, even for those having the same water activity. This affects the amount of water available in the micro-environment surrounding the microorganisms. For example, less water was required for rapeseed meal than for sugarcane bagasse to achieve an equivalent level of growth. Our conclusion is that the characteristics of the micro-environment have a much greater impact on growth than the macroscopic features of the whole substrate.

Our research based on microorganisms growing on solid substrates points to a new direction for studies focusing on solid state bioprocesses. It also adds a new layer of understanding to existing microbiology knowledge.

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O15-6

Microbial electrochemical technology (MET) platform for turning carbon dioxide into a suitable substrate for a chain-elongation fermenter

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Microbial electrochemical technologies (METs) have emerged as a promising technology to produce acetate and other organics from CO_2. This work aimed to perform not only acetogenesis, but also solventogenesis, at equimolar acetate/ethanol ratio in order to achieve an optimum influent for a chain-elongating fermenter.

With this objective, a two-chambered tubular bioelectrochemical reactor was inoculated with an enriched culture of isolate I-19 (tentatively, a Eubacterium limosum isolate), which previously showed a concomitant production of acetate and ethanol from syngas fermentation at 37 °C. The solventogenic reactor was fed with CO_2 as the sole carbon source and operated in batch mode at ambient temperature (25 °C). A 580 cm^2 cation exchange membrane separated the anode and cathode compartments. The biocathode (estimated surface of 0.59 m^2) was made of graphite granules and was operated at a fixed potential of −800 mV (vs. standard hydrogen electrode), whereas the sacrificial anode consisted of a carbon cloth (280 cm^2).

The biocathode averaged a concomitant production of $0.082 \pm 0.048\ g_{ethanol}\ L\ d^{-1}\ m^{-2}$ and $0.099 \pm 0.062\ g_{acetate}\ L\ d^{-1}\ m^{-2}$, demanding a current of $0.56 \pm 0.04\ A\ m^{-2}$ as average in 21 days of experiment. The most suitable concentration achieved in the catholyte was 1.15 and 0.96 $g\ L^{-1}$ of ethanol and acetate respectively. Moreover, changes in current density profiles were directly related to CO_2 depletion and transition from acetogenesis to solventogenesis, which demonstrates that C/e^- ratio may be a significant operational variable for the system.

In the light of the results reported, METs were confirmed as the initial of a two-step fermentation process through the production of an equimolar ethanol/acetate refined efflux to perform chain elongation for further C6–C8 production.

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Symposium 16: Applied Biocatalysis

O16-1

Efficient immobilization of α-naphtyl acetate esterase (ANAE) on modified k-carrageenan, and k-carrageenan/alginate composite matrices – a comparative evaluation

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Enzymes are protein that act as a catalyst to stimulate the biochemical reactions. By reducing the activation energy. However, free enzyme is expensive, unstable, difficult to separate and reuse thus limiting its applications in large-scale operations. Immobilization means confinement of enzymes usually in a semi-permeable matrix support to improve enzyme stability with respect to catalytic activity for repetitive use with the obvious ease of separation. This greatly increases the enzyme efficiency thus reducing the production cost in bioprocess industries. In this study, modified biopolymer such as cross linked pure k-carrageenan and k-carrageenan/alginate composite matrices are prepared as support for the immobilization of α-naphtyl acetate esterase (ANAE) extracted from wheat flour. FESEM and FTIR spectroscopy characterization was done for the novel support matrices. Optimum key parameters were identified for immobilization yield and enzymatic activity for two immobilization methods of entrapment and covalent bonding for the two novel support matrices developed. Maximum immobilization yield of about 90% of ANAE on k-carrageenan/alginate composite matrix using covalent bonding was obtained for 3% polyelectrolyte polyethylene imine (PEI) solution, 4 h curing time and pH 8. For entrapment method, the maximum immobilization yield obtained was 89% for 5 h curing time. The maximum enzyme activity was observed for the physically entrapped ANAE on composite matrix support compared to free and immobilized enzyme on other matrices. Optimum pH and temperature was found to be around 8 and 42 °C respectively for the composite matrix. Reuseability appeared superior for covalently immobilized enzyme while storage stability was better for entrapment method.

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O16-2

Enzymes and ice binding proteins from Antarctic organisms

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Living beings that thrive in habitats where temperature approaches the water freezing point have evolved different adaptive strategies, among these cold-active enzymes and ice binding proteins (IBPs), that are of interest for biotechnological applications [1]. Cold-active enzymes retain high specific activity at low temperature and are suited for low-temperature processes, i.e. detergency, food processing, transformation of heat labile compounds. Moreover, IBPs bind to ice crystals and inhibit their growth thus avoiding freezing and the formation of large ice crystals.

From a microbial consortium sampled in Antarctica and made up of the ciliate *Euplotes focardii* and symbiotic uncultured bacteria, we have obtained cold-adapted enzymes (superoxide dismutase, beta-galactosidase, lipase, lysozyme) and IBPs. We will discuss about the peculiar features of superoxide dismutases and a beta-galactosidase that couple activity at low temperature with a surprising stability. Moreover, we have characterized in depth a bacterial IBP endowed with peculiar ice activity and studied the structural basis of its behavior by protein engineering and X-ray crystallography [2,3].

This work was supported by the Progetto Nazionale di Ricerche in Antartide PEA 2014-2016 “Genome scanning and characterization of novel antifreeze proteins for industrial application” and by the RISE MSCA Project “Advanced bioinformatics for genome and metagenome analyses and discovery of novel biocatalysts from extremophiles: implications for improving industrial bioprocesses (MeTable)”.

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O16-3

Immobilization of carbonic anhydrase for enhancement of CO_2 reactive absorption

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CO_2 reactive absorption in aqueous solvents was proposed in the last decades as an affective process for post-combustion CO_2 capture. The use of the enzyme carbonic anhydrase (CA) as promoter of the reactive absorption process was studied and several options showed promising results in terms of CO_2 capture rate enhancement thanks to the effect of CA catalysis of the CO_2 hydration reaction to bicarbonate ions. The possibility to exploit this process at industrial scale for CO_2 capture from flue gases, directly from air, and from any other gaseous mixture (e.g. biogas) depends on the possibility to reuse and recycle the enzyme. CA immobilization is one of the most effective solutions to this issue. The aim of this work is to develop two different techniques of covalent immobilization of CA that may satisfy the requirements for effective enhancement of the gas-liquid absorption process. To this aim, a thermostable CA was immobilized in cross-linked enzyme aggregates (CLEA) and through covalent attachment on fine particles. In both cases, paramagnetic nano-particles (PNP) were used so that magnetic field assisted operations can be proposed for the end use of the developed biocatalyst particles in the CO_2 capture reactor. Commercial PNP were included during the preparation of CLEA and *ad hoc* synthetized PNP were used as supports for direct binding of CA through carbodiimide activation. Performances of both biocatalysts were assessed in terms of CO_2 absorption rate in a stirred cell lab scale reactor using K_2CO_3 solution as potential real solvents for CO_2 capture process.

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O16-4

Carboxylic acid reductase enzymes

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Carboxylate reductases (CARs) are emerging as valuable catalysts for the selective one-step reduction of carboxylic acids to their corresponding aldehydes. The substrate scope of CARs is exceptionally broad and offers potential for their application in diverse synthetic processes. Two major fields of application are the preparation of aldehydes as end products for the flavor and fragrance sector and the integration of CARs in cascade reactions with aldehydes as the key intermediates [1]. A current trend is to generate diversity in the CAR toolbox and various labs worldwide, including ours, contribute new enzymes and new strategies to incorporate them in reaction cascades.

In this study, we particularly aimed for a deeper understanding of CARs and their structure-function relationship and elucidated the role of approximately 20 highly conserved amino acid residues and their implication on catalytic activity and substrate preference [2]. In a second protein engineering approach, a random library of CAR mutants was screened with a newly developed high throughput assay that is specific for the aldehyde product [3]. Ultimately, our results will facilitate focused engineering of known protein scaffolds towards new CARs with outstanding properties such as thermostability or high activities for particular substrates.

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O16-5

Thermotolerant biotechnology: biocatalysts for added manufacturing

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Applications employing catalysts in fluidic, cascaded setup are becoming increasingly relevant, especially since the increasing use of added manufacturing technologies [1].

The objective of the research presented here is to enable the implementation of biocatalysts in added manufacturing. Such bioprinting applications call for thermotolerant organisms and proteins, which can be integrated into functional systems using thermoplasts with melting temperatures above 50 °C.

In our work we recently showed how we employ guided protein evolution for engineering biocatalysts for such applications [2]. To this end several aspects have to be addressed, amongst them selection of a promising starting point for the protein evolution and the ability to screen for activity under non-standard conditions. We are using a combination of several bioinformatics approaches to generate libraries of protein variants with improved thermostability as well as activity at increased reaction temperatures. Subsequently we developed a screen to analyze random mutant libraries. With this approach, we have successfully evolved enzyme variants that reveal significantly increased stability and activity at elevated temperatures up to 60 °C. These improved variants, along with naturally thermostable proteins, were then used for direct 3D printing of bioinks [2].

In this way, we could show for the first time the direct printing of enzymes inside the supporting material resulting in one-step fabrication of flow reactor modules. The flow reactors could be fabricated on demand from biokinks, which are stable over weeks inside the printing cartridge. Reactor modules containing different enzymes could be arranged into cascades and showed a tuneable behavior.

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O16-6

Production of short-chain fructooligosaccharides with a recombinant produced and immobilized fructosyltransferase

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In the wake of the growing trend towards consumption of so-called healthy foods short-chain fructooligosaccharides (scFOS) became attractive as low-calorie sweeteners. ScFOS consist of one glucose and up to six fructose units and can be differentiated by the glycosidic linkages and the oligomerization degree of the fructose moieties. Depending on the chain length, scFOS have a sweetness level of 30–60%, just as high as that of sucrose. However, scFOS cannot be digested by the human digestive system, but they can stimulate the growth of positive gut bacteria, making them a prebiotic nutrition constituent. In this study scFOS are produced with a new fructosyltransferase (FTase) derived from *Aspergillus terreus* and recombinantly expressed in *Kluyveromyces lactis* GG799. The recombinant FTase was produced in a 5L scale submerged fermentation in an adapted chemically defined medium. After cell removal the FTase was immobilized on two different epoxy resin carriers (Immobead 150P and Lifetech™ ECR8285). The carrier were compared with regard to the immobilization efficiency and the loss of activity. Up to 50% of the activity could be recovered in comparison to the total enzyme activity of the free enzyme solution. The immobilized FTase was used for FOS production in a 5 h batch reaction resulting a sucrose conversion level of 96% and scFOS yield of 80%. ScFOS molecules with a polymerization degree of up to five additional fructose units were obtained. Further experiments will focus on removing the accumulated side-product, glucose, which acts as a possible inhibitor of the catalysis.

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Symposium 17: Analytics and QC

O17-1

Improving drug product QC through noninvasive analytics

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Current quality control (QC) of drug products is based on inference, where a few units from each batch are analyzed invasively at the point-of-release, from which the quality of the entire batch is inferred. Inference-based QC will likely miss rare pre-release defects due to manufacturing errors, and any post-release defects due to product mishandling. The likelihood for such defects is heightened for complex drugs, such as biologics and nanomedicines. Noninvasive analytics make it possible to implement verification-based practice, where quantitative data are collected on every unit in a batch, from point-of-release to point-of-care. One noninvasive analytic, water proton NMR will be discussed, including its application to FDA-approved complex drugs.

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O17-2

An experimental study of a novel design of a LabDisk for separation of targeted tumor cells

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Circulating tumor cells are specific types of cancer cells that detach from the cancer tumors, enter the blood and are disseminated through other parts of the body. Centrifugal microfluidic devices are novel platforms for separating targeted cells such as cancerous cells without the need for an external pump. These lab-disk platforms can control the fluid flow by changing the RPM of the device rotation. In this project, two centrifugal microfluidic systems with the goal of separation of cancerous cells have been designed, fabricated and experimentally tested. Both passive and active methods of separation are investigated in this study for separating breast cancer cells as target cells from fibroblast cells as non-target cells. The passive microfluidic system is based on the inertial effects of the geometrical contraction-expansion features of the microchannel on the fluid sample. Based on the experimental results, the target cell recovery of ~72.1% is achieved at optimal rotational speed of 2400 RPMs in the centrifugal platform. In order to use the active separation method, the superparamagnetic Fe_3O_4 nanoparticles are attached to the target cells through the exclusive antibody-antigen bond. First, specific Ep-CAM antibody of the target cell was coated on magnetic nanoparticles through a chemical bonding process. Then, the target cells were exposed to these antibodies in order to form magnetic target cells. Finally, the magnetophoretic force exerted by the external magnetic field of a permanent magnet resulted in the desired separation. In these tests, ~90.2% of target cells were separated at optimal rotational speed of 1500 RPMs.

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O17-3

Selection and characterization of immunodiagnostics biomarkers from secretomes: Successful examples in *Schistosoma mansoni*, *Leishmania infantum* and *Trypanosoma cruzi*

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Omics sciences advances have unprecedentedly accelerated the generation of data. However, technologies for experimentation and validation of these molecular targets did not follow the same evolution. To increase our ability to select candidate proteins for diagnostics targets and tackle Neglected Tropical Diseases, our group has been using bioinformatics tools for mining big data and select proteins rich in B-cell epitopes. Subsequently, these biomarkers are validated as recombinant proteins heterologously expressed in bacteria and eukaryotic cells such as *Pichia pastoris*, *Leishmania tarentolae* and insect cells. Using secretome data from three human pathogens, *Schistosoma mansoni*, *Leishmania infantum* and *Trypanosoma cruzi*, it was selected proteins that are secreted by these parasites at bloodstream stages. ELISA assays with *S. mansoni* proteins (Sm10.3 and AQP) were reactive against antibodies in blood samples from patients with schistosomiasis and non-reactive against samples from healthy patients. The same immune response profile was observed with *L. infantum* proteins (one mitochondrial metallo-peptidase, Rab-1 and ADF/Cofilin). Recombinant peptidase in ELISA assays was able to differentiate all infected-dogs samples from healthy-dogs samples, detecting antibodies even in some

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animals previously diagnosed as healthy by commercial immunodiagnostic kits, but positive in molecular methods (PCR). This biomarker showed greater sensitivity compared to the methods currently used in Brazil. *T. cruzi*, metascapase and cruzipain are also being analyzed and previous data from these recombinant proteins from both bacteria and insect cells are demonstrating the potential for differentiation between samples from healthy patients and patients with Chagas' disease. In summary, our results indicate an applicable approach for selection and validation of biomarkers for immunodiagnostic via bioinformatics tools and production of recombinant proteins in different heterologous expression systems.

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O17-4

Revealing the complex composition of lignin and resulting products by ultra-high resolution mass spectrometry (FT-ICR MS)

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Lignin is an abundant plant polymer obtained as a co-product from the paper/pulp and bioethanol industries. Its highly aromatic composition can be an important source of platform chemicals and replace the ones derived from fossil fuels but it is very recalcitrant to break down. Ongoing research in the valorisation of lignin is underpinned by the analytical techniques able to provide information about the chemical composition of lignin, which depends on the plant species and the extraction methods applied to separate lignin from the lignocellulosic material.

We use ultra-high resolution mass spectrometry, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), a very sensitive technique that enables the elemental assignment of thousands of compounds present in the lignin sample, to characterise different commercially available lignin types (Organosolv and Kraft lignins, and lignosulfonates). In addition to this, we have used FT-ICR-MS to monitor the effects of a microwave pretreatment on lignocellulosic material as well as the compounds derived from the incubation of lignin with laccase. In-house Python scripts and graphical summaries supported and enhanced the analysis of large sets of data obtained by FT-ICR-MS. The results obtained showed a distinct chemical diversity between the different technical lignins analysed, in agreement with the differences between the chemical extraction methods. FT-ICR-MS data also provided an insight about the preferred substrates for laccases, which will support the ongoing research work in the area of valorisation of lignin via enzymatic processes.

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O17-5

Novel microfluidic and analytical approaches for screening of engineered strains: Micro-sensor technologies for pH and organic acids detection

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Yeast fermentation products contain multiple ingredients such as succinate, pyruvate, and acetate; and high performance liquid chromatography (HPLC) is commonly applied to the monitoring of these compositions. However, applying lab-scale fermentation for optimizing the process or screening high-yield strains can be time consuming, labor intensive, and sample devouring. Microfluidic platforms are currently more favorable setups for early-stage screening and optimization. Nonetheless, the minute sample amount in microfluidic setup is challenging for HPLC analysis. Therefore, this study focuses on the development of micro-sensors for rapid and high dynamic range detections not only in performing the monitoring of the metabolic products (lactic acid, ethanol, etc.) and the substrate (glucose) but also the pH environment in the micro-scale fermentation. The micro-sensors utilized the Ni/Au electrode for detecting metabolic products and substrates while the InO_x electrode for pH measurements. The lactate detection had a detection range of 0.5–20 mM and a sensitivity of 65.94 μA/mM. For ethanol detection, the detection range was 1–20 mM and the sensitivity was 22.58 μA/mM. The glucose detection had a detection range of 0.5–20 mM and a sensitivity of 166.86 μA/mM. The pH sensor had a detection range from pH 2 to pH 10 with a sensitivity of 61.90 mV/pH. All detections were highly linear with correlation coefficients higher than 0.97. Up to now, pH sensors were tested during cultivations in microfluidic devices of a lactic acid-producing *S. cerevisiae* engineered strain to evaluate the pH change in the microliter-scale growth chamber resulting from the organic acid production.

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O17-6

A nanoparticle platform for accelerated surface-mediated assays of protein stability

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The stability of therapeutic proteins during bioprocessing and formulation is a crucial property to guarantee developability. In addition to assessing their activity, identifying stable molecules since the early stages of the process is important. Air–water and solid–liquid interfaces are well known to potentially trigger protein instability and aggregation. Here, we develop an accelerated stability assay providing a highly controlled surface-mediated driving force for aggregation under stagnant and shaking conditions. The platform consists of polymeric nanoparticles with different surface properties. The resulting high surface-to-volume ratio and flexibility of polymer chemistry allow to accurately control both the total area and the chemistry of the surface exposed to the proteins. We applied this high-throughput assay to investigate the stability of human insulin, a model IgG and BSA. In particular, we demonstrate the potential of this assay by investigating the combined effect of surface and hydrodynamic flow. We show that hydrophobic surfaces induce the formation of amyloid fibrils from soluble human insulin by specifically promoting the primary heterogeneous nucleation rate. In contrast, mechanical forces accelerate the formation of amyloid fibrils by favoring mass transport and amplify the number of fibrils by promoting fragmentation events. Thus, surfaces and agitation have a combined effect on the kinetics of protein aggregation observed at the macroscopic level, but individually, they each affect distinct microscopic reaction steps. These

results suggest that the inhibition of surface-induced heterogeneous nucleation should be considered a primary target to suppress aggregation and explain why in many systems the simultaneous presence of surfaces and hydrodynamic flow enhances protein aggregation [1].

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Symposium 18: Biodegradation and Bioremediation

O18-S

Organohalide respiration with chloroethenes: From fundamentals to application

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Chlorinated solvents such as per- and trichloroethene (PCE/TCE) are among the most frequently encountered groundwater pollutants due to their widespread use in industry and dry cleaning of cloths. Bacteria able to use these pollutants as terminal electron acceptor in an anaerobic respiration process, so-called organohalide-respiring bacteria (OHRB), are present in many natural environments. They can convert PCE and TCE to ethene by reductive dechlorination, however, the intermediate vinyl chloride often accumulates in aquifers where spontaneous dechlorination occurs, a compound which is much more toxic than the parent compounds PCE and TCE. The genomes of several OHRB have been sequenced and show that they can contain multiple putative reductive dehalogenase genes for which the substrates are not known. We try to unravel the substrate spectrum of these enzymes with an innovative biochemical approach creating hybrid proteins containing unknown and known parts of reductive dehalogenases. This will help to increase our knowledge on which chlorinated compounds could be treated by bioremediation. In addition, it will be shown how known knowledge on OHRB and reductive dehalogenases can be used to explain intermediate accumulation phenomena observed in different aquifers, and how one could even envisage reductive dechlorination as bioremediation process in source zones where acidification by fermentation and dechlorination is a major drawback for organohalide respiration to decontaminate such zones.

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O18-1

A semi-passive biofiltration process for the degradation of recalcitrant naphthenic acids from oil sands process waters

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With the utilization of indigenous microorganisms, a biologically active sand filter was set up and operated at the bench-scale level for the treatment of oil sands process water (OSPW). Quantitative polymerase chain reaction (qPCR) and confocal laser scanning microscopy (CLSM) showed that indigenous microorganisms were able to attach to and form a biofilm on the filter media. The number of total bacteria on the filter media reached a steady state (10^9/g) after a continuous operation of the biofilter using OSPW for a period of three weeks. After 87 days of operation, the biofilm thickness was 35.8 ± 0.9 μm. Following the initial acclamation/establishment period of the biofilter, removal efficiencies of 21.8% of classical naphthenic acids (O_2-NAs; initial concentration of 13.06 mg/L) and 12.9% of oxidized NAs (O_4-NAs; initial concentration of 6.13 mg/L), respectively, were achieved after eight times of re-circulation with a total hydraulic retention time (HRT) of 16 h. Following an ozonation pretreatment (utilized ozone dose of 30 mg/L), 89.3% of O_2-NAs could be removed from the ozonated OSPW with the HRT of 16 h. In addition, the biofiltration showed 52.9 and 42.6% removal efficiencies of O_3-NAs (initial concentration of 7.17 mg/L) and O_4-NAs (initial concentration of 6.13 mg/L), respectively, from the ozonated OSPW. Metagenomic sequencing analysis showed that Rhodococcus from the Actinobacteria phylum was the dominant bacterial genus on the sand media, playing an important role in the NA biodegradation. The combination of ozonation and biofiltration showed excellent NA removal efficiency, making it a promise reclamation strategy for OSPW.

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O18-2

Treatment of wastewater contaminated with atrazine using a 10583 packed bed reactor packing with an organic biomixture

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The packed bed reactor is considered a good wastewater treat-ment system due to organic pollutants removal through adsorption and degradation process. Therefore, the aim of the present work was to evaluate the operational conditions of a packed bed reactor using an organic biomixture to wastewater treatment contami-nated with atrazine in continuous system. The reactor was made of high quality glass material (15 cm × 8 cm) packed with an organic biomixture. The stock solution was prepared with commercial atrazine. Effect of pH (4, 6 and 8), atrazine concentration (5, 10, and 15 mg L−1) and flow rates (10, 30 and 50 mL h−1) were evaluated. Atrazine concentration and degradation products in the effluent were analysed by HPLC. Data obtained were evaluated through the surface response methodology (RSM). From the results obtained it was demonstrated that the most important factor in the pro-cess was pH. Because, to acidic pH (pH 4), atrazine removal was greater (>90%) without significant differences ($p < 0.05$) accord-ing to concentration and flow rate analysed. While, at alkaline pH (pH 8) atrazine removal was lowest (19.96%). The adsorption pro-cess is more significant at pH 4 than pH 6 and 8, with optimum result of 152.22 mg atrazine adsorbed per gram of biomixture under operation conditions: pH 4, 15 mg L−1 of atrazine and flow rate of 50 mL h−1.

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O18-3

Microbial and enzymatic approach for the reduction of terpenes in pinewood

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Terpenes are among the main sources of volatile emissions in the wood processing industry. Especially long-term exposi-tion to volatile organic compounds (VOC) is suspected to cause harmful conditions in indoor environments. The aim of this study therefore was to lower the total emission level of pinewood, the basic raw material for oriented strand boards (OSB), by applying terpene degrading microorganisms or enzymes onto wood. The main focus was laid on the three major terpenes in pine wood: α-pinene, β-pinene, and Δ 3-carene. While both pinenes were effi-ciently degraded by specifically selected and adapted Pseudomonas strains, Δ3-carene appeared to resist degradation. Therefore, we applied the fungus *Penicillium nigricans* in combination with the bacteria strains and accomplished for the first time a simultaneous reduction of the three major pinewood terpenes including Δ3-carene. Degradation rates for Δ3-carene were 80% and 30% in 4 and 2 days, respectively. In order to boost Δ3-carene degradation to a level that meets industry demands, we attempted to decompose Δ3-carene by oxidation using the oxidoreductase laccase isolated from the white-rot fungus *Trametes pubescens* as a biocatalyst. Laccase achieved an almost complete oxidation of Δ3-carene in defined liquid medium and a 30% reduction in pinewood particles after 24 h of incubation at optimum conditions. In liquid culture, carenones (car-3-ene-5-one, car-3-ene-2-one and car-2-ene-4-one) were detected as final metabolic products, which were also found in other research studies. These results provide a solid basis for future studies and integration into the industrial OSB production process.

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O18-4

Ability of *Fusarium culmorum* to degrade the endocrine disruptor di(2-ethyl hexyl) phthalate: Enzymes production and pathway of biodegradation

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Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer widely used in the manufacture of plastics, and it is an environmental contaminant. The presence of this compound in the environment as a pollutant raises concern because of its endocrine-disrupting toxicity. *Fusarium culmorum* has the ability to produce esterase enzymes. Esterases are of great importance because they can break the ester bonds present in the plasticizers. Biodegradation of DEHP by *F. culmorum* and its induction of esterases were studied. *F. culmorum* was grown on media containing DEHP (initial concentration 1000 mg/L) as sole carbon source at 25 °C for 8 d in submerged fermentation. Growth kinetics and esterases activity characterized by biochemical tests and polyacrylamide gel electrophoresis were evaluated. Biodegradation constant of DEHP (k), half-life of DEHP biodegradation ($t_{1/2}$) and percentage of removal efficiency ($\%E$) were also determined. Intermediate compounds of biodegraded DEHP were identified by GC-MS and a DEHP biodegradation pathway was proposed. *F. culmorum* degraded 100% of DEHP after an incubation period of 144 h. $\%E$, k and $t_{1/2}$ were 99.9, 0.0256 h^{-1} and 27 h, respectively. DEHP was metabolized to mono ethyl hexyl phthalate and ethylhexanol. Maximum esterase activity was 845 U/L and esterase activity bands were observed in the DEHP-supplemented media, having a molecular weight of about 75 kDa. *F. culmorum* has a promising ability for bioremediation of environments polluted with DEHP because it efficiently degrades DEHP and

uses high concentrations of this compound as carbon and energy source.

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O18-5

Isolation and identification of erythromycin-mineralizing bacteria

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Antibiotics are widely used in human and veterinary medicine to treat and prevent bacterial infections. Often being only partially removed in wastewater treatment plants (WWTP), they are released into the environment with the treated effluents. The presence of antibiotics is associated with the development of resistant bacteria, which poses a public health risk. Due to its frequent presence in WWTP effluents, the antibiotic erythromycin is on the EU Watch List for emerging water pollutants.

The aim of this study is to isolate and identify erythromycin-degrading microorganisms. Activated sludge from a local WWTP was incubated in semi-continuous sludge reactors. Weekly, the medium in the reactors was exchanged by fresh mineral medium containing erythromycin (1 mg/L) and yeast extract (0.5 g/L). After six weeks of incubation, a dilution series was incubated with ^{14}C-[N-methyl]-erythromycin to allow for liquid scintillation counting of $^{14}CO_2$ formed from erythromycin by microbial metabolism. Further dilution series were set up when apparent partial mineralization reached 50% in diluted samples. In the course of eight dilution series, we decreased stepwise the concentration of yeast extract to 0.005 mg/mL, while increasing the concentration of erythromycin to 100 mg/L to select strictly for erythromycin degraders. Currently, the isolation of strains from these enrichment cultures by agar plating and the verification of their ability to degrade erythromycin are ongoing.

Results from this study will contribute to a better understanding of the processes involved in the degradation of erythromycin during the wastewater treatment process.

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O18-6

Biological recycling of metals contained in lithium-ion batteries (LIB)

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The need to replace fossil fuels with cleaner alternatives with a lower carbon footprint has led to an increasing popularity in the use of electric vehicles (EV). Lithium-ion batteries (LIB) are one of the most common forms of energy storage in EV and electronic devices due to their advantages over other battery types, such as being smaller and lighter with higher energy density. LIB are considered a safe and clean technology; however, due to their high demand, there are environmental and socio-economic concerns over the scarcity of the raw materials required, accumulation of waste at the end of the LIB life cycle, and the hazards associated with the metals contained in the spent LIB. In this context, the aim of the present project involves the bioremediation and up-cycling of metals contained in LIB at the end of their life. Current work is being developed with two different bacterial strains from the *Desulfovibrio* and *Morganella* genera. The tolerance of these two species to relevant metals is being investigated together with their ability to reduce metal bioavailability and the production of valuable nanoparticles from waste material. Synthetic biology tools are being developed in parallel to enable the domestication of these organisms and to improve their applications for bioremediation and nanoparticle synthesis purposes.

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Symposium 19: Biobased Resources and Biorefineries

O19-1

The challenge of producing bioethanol from residues of Kraft Pulp processing

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Residual lignocellulosic biomass emerge as sustainable alternative for fossil resources, the main source for the production of energy, chemicals and materials.

Agroforestry is one of the most important sectors of the Portuguese economy with the pulp and paper industry being a major contributor to the growth of the balance of trades. Paper pulp is composed essentially by cellulose and few hemicelluloses. These polysaccharides can be converted into fermentable sugars, by the action of hydrolytic enzymes like cellulases. These sugars may be metabolized by microorganisms into second generation biofuels. Compared to other second generation processes this is an advantage since lignocellulosic biomass (LCB) is recalcitrant and has a varied chemical composition and physical characteristics. There are some technical and economic challenges for the conversion of LCB to biofuels, since a costly pre-treatment is required.

Pulp and paper Kraft pulping process removes lignin and targets hemicelluloses and can be exploited as pre-treatment for subsequent bioethanol production. So, the pulp and paper industry can be converted into integrated biorefineries increasing the opportunity of success of the bioethanol production.

The aim of this study was to investigate the production of second generation bioethanol from Eucalyptus globulus unbleached Kraft pulp, exploiting the Kraft pulping process as LCB pre-treatment. This work will help to access the feasibility of producing bioethanol from pulp and paper industry wastes, such as low quality wood, bark and other rejects, and low quality and excess pulp, so that an integrated biorefinery can be implemented in the existing Kraft pulp mills.

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O19-2

Biopolymer production by a halophilic bacteria in an integrated biorefinery

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There is a strong trend towards the use of sustainable, biodegradable and renewable materials. Among all the known biopolymers that can replace the petroleum-based polymers, polyhydroxyalkanoate (PHA) stands out for many applications due to its special features. PHA production is a well-known process that had kept much attention, however, several issues that affect the production costs threaten its industrialization. An approach to overcome this problem is the integration of PHA production into ethanol biorefineries. In this study, several PHA production strategies framed within a biorefinery scheme have been developed based on the use of the halophilic bacteria *Halomonas boliviensis*. Halophilic microorganisms have a great potential in biotechnological applications, but they have been scarcely used at industrial scale.

The PHA production strategies include the use of (a) process streams from different biorefinery feedstocks (cereal mash, sugarcane, etc.), (b) volatile fatty acids, which may be obtained from the anaerobic treatment of the biorefinery wastewater, and (c) the integration of PHA synthesis in a simultaneous saccharification and fermentation (SSF) process. The PHA production obtained using cereal mash and following an SSF strategy at bioreactor scale (26 g/L) was in the range of the best results previously obtained with complex substrates. All these contributions are oriented to develop the future sustainable production of PHA within a biorefinery.

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O19-3

Multi-objective optimization of biobutanol production using surrogate models

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There is a significant development in the production biofuels from renewable resources. Recent decades have witnessed a major increase in the production of bioethanol and biodiesel. More recently, biobutanol, which is considered a better biofuel, has been the subject of increased attention. The biobutanol production process, referred to as the Acetone–Butanol–Ethanol (ABE) fermentation, has major limitations to overcome before becoming economically viable.

Microorganism genetic manipulation and ABE solvent in situ recovery are used to partly address these limitations. This investigation considers the continuous ABE fermentation process integrated with membrane pervaporation to extract part of the ABE solvents to partly mitigate product inhibition. A phenomenological model of the integrated ABE process has been developed and used to optimize the overall process. The model was used to obtain the Pareto domain of the whole process considering four objective functions (butanol productivity, overall butanol concentration, sugar conversion and membrane area) and five decision variables (dilution rate, inlet sugar concentration, cell retention factor, number of membrane modules in series and the number of stacked membranes in each module). To reduce the huge computation time to circumscribe the Pareto domain, artificial neural network (ANN) surrogate models for the four objectives were derived with a small input data set selected with uniform design and then used in the optimization genetic algorithm. The inputs of the ANNs were the five decision variables. The surrogate models led to an accurate Pareto domain and reduced the computation time to circumscribe the Pareto domain by a factor of 2500.

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O19-4

Engineering carbon-conserving synthetic pathways for assimilation and conversion of C5/C6 carbon sources into added value chemicals

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The development of carbon efficient pathways for added value (bio)chemicals production is the essence of White Biotechnology. The limit of carbon conservation in all (bio)chemical syntheses is determined by the electron balance in substrate(s) and product(s). Natural pathways do not have often the stoichiometric capacity to produce a value-added compound at yields that correspond to the thermodynamic maximum. A good example of this lack of stoichiometric efficiency is the bioproduction of glycolic acid (GA), a two carbon compound of considerable industrial interest notably in cosmetics and biodegradable polymers. We addressed this objective by employing the following strategies. Firstly, we reconsider a new pathway for C5 assimilation that relies on the carbon-conserving aldolytic cleavage of X1P or R1P to yield the C2 compound glycolaldehyde and the C3 DHAP compound by expression of a ketohexo-1-kinase (Khk-C) and aldolase (Aldo-B). Glycoaldehyde is then either reduced into ethylene glycol or oxidized into glycolic acid usingh endogenous reductase/dehydrogenase. With this approach, EG and GA at yield close to maximal of 1 mol/mol sugar have been obtained. This synthetic pathway was then combined with the natural glyoxylate shunt, to yield a production of GA ~30% from a xylose/glucose mixture (66%/33%) 30% higher than using the sole natural pathway. Yet, as this strategy does not avoid a loss of carbon at the level of pyruvate, we created a cycling route that overcome this loss and which allowed both hexoses and pentoses to be converted into glycolic acid at their highest theoretical yield.

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O19-5

Optimising nature for industry: Design of synthetic promoters for strain engineering of *Trichoderma reesei*

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Over the recent years a lot of effort and research have been made to develop the fossil fuel-based chemical industry towards a bio-based industry. Trichoderma reesei is a filamentous fungus that is well established for the production of biorefinery enzymes, mostly cellulases and hemicellulases. To optimise the production, different genetic tools are necessary to optimise yields.

The promoter of the uncharacterized gene cdna1 (Pcdna1) is one of the strongest promoters that is active during growth on glucose. Although stronger promoters exist, they are often repressed on glucose or require activation by specific inducers. Our goal was to rationally design a promoter as strong as an inducible promoter but active during growth on glucose.

We analysed 50 highly expressed genes towards their core promoter structure. Based on the findings, we established a box structure system where 6–20 bp long sequences (boxes) were arranged on a 200 bp long backbone. Eight different artificial sequences were designed and fused to the endoglucanase CEL12A as a reporter. We found that two of the synthetic core promoters surpassed the expression strength of the native Pcdna1 by about 175%. The design of the artificial core promoters included already known sequences like TATA-boxes, GC-boxes or CT-rich regions, but also newly found boxes from our sequence analysis.

We correlated the core promoter structures to the expression levels and suggest using our experimental data for classifying and identifying new promoters suitable for genetic engineering.

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O19-6

Consolidated bioprocessing in engineered *B. subtilis* lab strains: γ-PGA production from biomass

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The soil bacterium *B. subtilis*, the model organism for Gram positive bacteria, is the best characterized member of the *Bacillus* genus, which includes several highly exploited industrial species. Besides industrial enzymes, *Bacillus* spp. can synthesize poly-γ-glutamic acid (γ-PGA), a nontoxic, biodegradable, highly anionic biopolymer made up of multiple D-/L-glutamic acid monomers joined by amide linkages between the α-NH2 and γ-COOH groups. Thanks to several valuable characteristics, γ-PGA is applied in an expanding

range of biotechnological fields (Ogunleye et al., 2015), including applications as drug carrier, gene delivery and scaffold material for tissue engineering (Luo et al., 2016). γ-PGA production is also an ideal model system to develop cost-competitive feedstocks for *B. subtilis* aerobic fermentations.

Despite *B. subtilis* possessing a wide array of complex-carbohydrates degrading enzymes, direct transformation of biomass into biocommodities has not yet been reported for this microorganism.

The aim of this work was to obtain economic γ-PGA production using a waste biomass as feedstock. Rice straw is one of the most abundant biomass resources, not in competition with food, for which there are no effective valorisation strategies.

In this study, the cellulolytic capabilities of *B. subtilis* JH642 were maximized through self-cloning procedures, and a cheap and simple pretreatment to facilitate straw saccharification was developed.

The engineered strain grew efficiently on treated straw. Moreover, by transferring the mutations supporting γ-PGA biosynthesis (Scoffone et al., 2013) into the cellulolytic strain, direct production of γ-PGA from biomass was obtained, definitely proving the applicability of Consolidated Bioprocessing concepts to *B. subtilis*.

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Symposium 20: Nanobiotechnology (jointly with AfoB)

O20-1

Biotransformation of copper nanoparticles for remediation of distillery co-products

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We are investigating the bioremediation of whisky distillery co-products using the metal resistant bacterium *Morganella psychrotolerans*. Copper vessels must be used in whisky distillation as the copper plays a crucial role in the creation of the spirit, which once matured becomes Scotch Whisky. The use of copper pot stills in the production of Scotch Whisky is also enshrined in law as part of the Scotch Whisky Regulations (2009). This means pot ale and spent lees, the co-products left over from distillation, contain a low concentration of copper ions. Pot ale contains nutritious yeast and grains, making it a good fertiliser, but the copper must be removed before it is used to avoid it building up in the environment. We aim to perform this remediation biologically, and are engineering *M. psychrotolerans* to convert the copper into useful nanoparticles.

To this end, we have used proteomics to investigate protein expression levels in *M. psychrotolerans* in the presence of copper to identify targets in the nanoparticle production and copper resistance pathways. We have cloned a number of these targets in order to assess their effect on copper resistance and nanoparticle synthesis when overexpressed, and their potential for aiding copper removal. We have also developed CRISPR-cas9 tools for the deletion of key genes, to better understand and control this process.

Using this we aim to increase nanoparticle yield and make use of biological factors for stability and nanoparticle modification.

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O20-2

Fast, simple and affordable access to space for biotechnology R&D through ICE Cubes service

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Gravity is a pervasive force that influences all aspects of life on Earth. By removing the effects of gravity, it is possible to study life functions and processes down to the cellular level in plants, ani-mals and humans. It enables researchers to observe and control phenomena that are normally masked by effects of gravity, and to perform experiments that would be impossible on Earth's surface. The International Space Station is an extraordinary microgravity laboratory and creates a research environment where processes can be observed without the distortions experienced on Earth. Biotechnology R&D can benefit significantly from experiment-ing in space through the deeper understanding of biological systems which are affected by microgravity through a vast array of changes such as: global alterations in genes' expression, molec-ular signaling networks change, cell metabolisms modification, cell growth acceleration, tissues regeneration rates decrease, microor-ganisms virulence increase, 3-dimensional aggregation of cells into tissue-like architecture. Space access is no longer restricted to agencies and governments only, but a new commercial spaceflight era has started. Based on a partnership with the European Space Agency, the International Commercial Experiment Cubes (ICE Cubes) service has been estab-lished as first such service in Europe to provide fast, simple and affordable access to space for research and technology. Scientists and industries are more and more searching for answers in space and obtain results that push back the frontiers of understanding on Earth. New biotechnology discoveries can be achieved in space through the ICE Cubes service, transform-ing basic research findings into practical applications, such as new medicines or therapies, new (bio)-materials as well as innovative environmental, agricultural and industrial processes, technologies and products.

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O20-3

Bioelectronic nose based on olfactory receptors and carbon nanotube devices

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Extensive efforts have been given to develop artificial sensory devices which can imitate the responses of human noses and tongues using solid state electronics. However, such devices based on solid state electronics are usually inferior to human sensory systems in terms of its sensitivity and selectivity. In human olfactory systems, olfactory receptor molecules can selectively bind to

specific odorant molecules, which allows humans to distinguish specific smells with a high sensitivity. In our work, we coated *olfactory* or *taste* receptors on carbon nanotube-based transistors to build bio-electronic *noses* or *tongues* which can imitate human sensory systems, respectively. In this device, when specific molecules bind selectively to the receptor molecules, localized charges are generated in the receptor molecules and alter the conductance of the underlyng carbon nanotube devices. Thus, one can selectively detect odorant or taste molecules simply by monitoring the currents in the underlying carbon nanotube devices. In this presentation, we will discuss the bioelectronic nose and tongues based on carbon nanotube devices and receptor proteins. Future prospects and possible applications of these devices also will be discussed.

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O20-4

Novel microfluidic and analytical approaches for screening engineered strains: Downscaling of *S. cerevisae* cultivations for lactic acid production

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The development of a biotechnological process depends on the optimization of a number of parameters, such as the strain, the cultivation mode, pH, temperature, dissolved oxygen concentration, growth behaviour and others. Establishing the right protocol requires a huge amount of work and time, therefore it is not surprising that there is an increasing demand for novel systems able to perform parallel processes and high-throughput analyses.

Microfluidics – and the miniaturization of laboratory instruments in general – has become a powerful tool for biotechnology and its applications have constantly increased in the last decade. This project aim is a multi-module microfluidic platform with a cultivation system and integrated sensors for operational and product parameters monitoring able to screen multiple strains. Small-scale devices provide us with the opportunity to have a better control of the whole experimental system and to reduce cost in the early stage of industrial process development.

Up to now, the basic module of the platform has been designed and fabricated. The cultivation chamber is made of PDMS (Polydimethylsiloxane) and can be used under both batch mode and perfusion mode conditions. The first experiments have been performed with the model system *Saccharomyces cerevisiae* (both wild type and a lactic-acid producing engineered strain) which could efficiently be grown both in batch and perfusion mode. Three sensing methods have been characterized and proved to be effective at detecting dissolved oxygen, biomass and pH during experiments on chip. All these strategies are non-invasive and they allow to continuously acquire data about the process occurring inside the chamber without the need of sampling.

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O20-5

Withdrawn

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Symposium 21: Formulation, Package and Delivery

O21-1

Development of amphiphilic dextran-based nanoparticle and *in vitro* cytotoxicity in human colon adenocarcinoma cell

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Nanoparticles are widely used in various applications such as packaging materials for food, nutrient encapsulation in cosmetics, and drug delivery in therapeutic purpose. In pharmaceutical perspective, nanoparticle can be applied as a carrier of therapeutic molecules to provide protection, enhance stability, and increase efficiency of those molecules. Along with other natural materials, dextran (Dex) is recognized for its biodegradable, biocompatible, and low cytotoxic properties. However, alteration of dextran hydrophobicity is required in order to allow self-assemble nanoparticle formation. In this study, the long alkyl chain esters of vinyl laurate and vinyl decanoate were added to dextran by lipase-catalyzed transesterification reaction to obtain amphiphilic dextran. Both dextran laurate ester (Dex-L) and decanoate ester (Dex-D) were characterized and used for nanoparticle formation. Modified dextran nanoparticles were prepared by nanoprecipitation followed by dialysis for Dex-L, and nanoprecipitation followed by solvent evaporation for Dex-D. The results from TEM and DLS suggest that both Dex-L and Dex-D nanoparticles were in spherical shape with average size distribution of less than 200 nm in diameter. The low cytotoxicity effect on cell viability of both modified dextran nanoparticles were observed *in vitro* in human colon adenocarcinoma cell line using MTT assay. Our results provide an alternative approach in synthesizing dextran-based nanoparticles that can be further developed for biomedical application.

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O21-2

A platform technology for the bioconjugation of nanoparticles in cancer theranostics

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The coupling of nanoparticles (NPs) to cancer-targeting biomolecules (e.g. antibodies) is fundamental to their use in cancer theranostics. However, conventional bioconjugation techniques such as physical adsorption or cross-linking often lead to the attachment of biomolecules with altered conformations and random orientations causing a reduction/loss of function.

We have established a versatile bioconjugation platform technology based on a peptide (referred to as the 'Linker') that binds with nanomolar affinity to a range of silica materials. The linker (L-) sequence can be genetically fused to a protein of interest and the resulting recombinant fusion protein (L-Protein) exhibits strong binding to silica. Herein, the linker was fused to either (i) Protein G (PG), which binds antibodies, or (ii) Barstar (Bs), which binds proteins tagged with its binding partner Barnase (Bn). The fusion proteins L-PG and L-Bs, mediated the orientated immobilisation of antibodies or Bn-tagged proteins onto silica surfaces, respectively. This bioconjugation occurred within minutes and without the need for any complex chemical reactions.

Using L-PG and L-Bs, antibodies or Bn-tagged proteins that target cancers were attached to the surface of silica-coated NPs with differing modalities (i.e. fluorescent dye-doped, lanthanide-doped upconversion, and superparamagnetic). These functionalised NPs were successfully applied in the targeted imaging of a variety of cancer cell types, including brain, breast, colorectal, and bladder cancers. Additionally, the functionalised NPs remained stable and retained functionality in complex biological fluids such as mouse whole blood. Thus, this unique and robust bioconjugation platform shows promise for *in vivo* cancer theranostic applications (e.g. drug delivery).

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O21-3

Anti-angiogenic strategies for chemoembolization of liver tumors local controlled delivery of antiangiogenics for liver cancer treatment

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A clinical option for the treatment of hypervascular solid tumors is the embolization of the tumor tissue by injection of hydrogel beads, obstructing the vascular bed of the tumor, leading to ischemic necrosis. These beads may additionally release chemotherapeutic agents in the tumor tissue – a procedure called TACE, transarterial chemoembolization. A pitfall of the procedure lies in the hypoxia-induced release of pro-angiogenic factors, eventually leading tumor recurrence. The delivery of antiangiogenic drugs to the tumor tissue would therefore prevent the tumor from growing new blood vessels, improving the efficacy of the treatment.

In this study, the effective combination of the multi-tyrosine kinase inhibitor sunitinib with hydrogel microspheres was demonstrated *in vitro* by loading and release studies. *In vivo* data acquired in a rabbit tumor model support the antitumoral effect as well as the ability of the local delivery approach to sustain high sunitinib concentration at the tumor up to 14 days following the intervention, while keeping plasma concentration below therapeutic levels.

Local treatment with antiangiogenic may thus provide a safe, promising approach for the treatment of liver cancer. Delivery of various anti-angiogenics may be considered, from vandetanib – under clinical investigation – to biopharmaceutics such as Bevacizumab, providing new tools to the interventional oncologist.

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O21-4

PLGA microparticle carrier system as adjuvant approach for pandemic influenza vaccines; Proof-of-principle with adsorbed whole inactivated H5N1 influenza

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For the development of pandemic vaccines, cationic poly lactic-co-glycolic acid microparticles (PMPs) have been evaluated as an antigen carrier system for whole inactivated influenza (wH5N1).

Depending on the nature of the cationic reagent, branched polyethylenimine (PEI) or DEAE-dextran (DEAE), it can be added to different phases in the manufacturing method. Through optimization, two microparticle formulations were developed with a surface charge between 15-30 mV and a particle range of 20–30 μm. The adsorbed virus, previously labeled by a fluorescent lipid probe Octadecyl Rhodamine B Chloride (R18), was detected by confocal imaging. The immunogenicity of the carrier system was assessed *in vivo* by serum hemagglutination inhibition (HAI). An initial peak (HI titer 185) is attained with the PMP-DEAE formulation, and then a stable HI titer of 25 is measured up to day 105, comparable to a single administration of wH5N1. *In vivo* results indicate the interactions with PMP surfaces have not affected WIV antigenicity.

The immediate and potent response measured is desired in a pandemic situation, indicating the formulation has potential for further development. The current proof-of-concept can be further elaborated with the aim to obtain broad cross-protective immunity.

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O21-5

Modeling the physicochemical properties of DNA:PEI polyplexes on transient gene expression: A DoE-based approach

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Polyethylenimine (PEI)-mediated transfection is a generalized system for the expression of a handful of recombinant proteins in animal cell lines. The diversity of protocols used for PEI-mediated transfection has revealed a number of variables affecting the yield of this methodology [1]. Thereafter, the impact of different parameters such as ion concentration, pH or incubation time has been individually evaluated [2–4] but synergies between these variables on the DNA:PEI polyplex formation (i.e. morphology, size or aggregation) are still unknown.

This work focuses on the characterization of the DNA:PEI complexing process in an incubation solution with known composition towards defining a robust transfection condition for HEK293 mammalian cells. To do this, several complexation solutions have been evaluated and NaCl has been selected as the best in terms of Virus-Like Particle (VLP) production. Statistical designs have been combined to generate a model describing both the transfection efficiency and VLP production depending on the NaCl ion strength, pH and incubation time of DNA and PEI. The optimum condition considering these two responses is 125 mM of NaCl concentration, pH 7 and 10 min of incubation.

Cryo-electron microscopy, Dynamic Light Scattering and Nanoparticle Tracking Analysis have been applied to better characterize and quantify the DNA:PEI complexes in different conditions. It is observed that below 150 mM of NaCl, DNA and PEI aggregate more rapidly in the form of branched-like structures compared to higher NaCl concentrations.

The results presented here offer an advance in the comprehension of PEI-based transfections where DNA:PEI polyplex properties are not fully understood yet.

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O21-6

Using some lyoprotectants for shelf life improvement of a lyophilized intravesical immune BCG

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Intravesical immune BCG (Bacillus Calmette-Guerin) as an immune response modifier against superfacial bladder cancer currently is being produced in Iran in form of suspension and ready to use at once but also requires storage and distribution in freeze state with extra care and cost. Since lyophilization successfulness is a strain and technical dependent process, in order to overcome this weakness, efficacy of some admissible LPs upon biological and physical characteristics of BCG (strain:1173P2) bacilli was investigated to provide a more potent lyophilized product through, after lyophilization and during long storage period. Bacterial bulk was formulated with different amounts of some lyoprotectants including sucrose, lactose, trehalose, glucose, dextran-40, and monosodium L-glutamate (MSG) and submitted to freeze drier. Furthermore lyophilizationcycle was optimized for the best results. Characteristics have been significantly improved by a lyophilized sample contained a combination of lactose, MSG, dextran-40 and tween-80 so that the satisfied results for viability, moisture content, appearance, reconstitution time, and shelf life period were achieved

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Symposium 22: Sustainable Environmental Technologies

O22-1

Targeting the Torso signalling pathway to identify bee-friendly insecticides

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Bees are economically important pollinators and their declining populations pose a threat to agricultural productivity. Mounting evidence points to pesticide toxicity as a cause for colony collapse and new approaches for identifying selective insecticides that achieve targeted control of insect pests with minimal effects on bees are needed. Embryonic terminal patterning and moulting are critical developmental processes in insects which are regulated differently in pests and bees. In pests, both these processes occur downstream of a MAP kinase signalling pathway triggered by activation of a common receptor Torso, by the ligands Trunk and Prothoracicotropic hormone (PTTH), in the respective developmental contexts. In contrast, bees do not have orthologs for Torso, Trunk or PTTH encoded in their genomes and terminal patterning is carried out through a different pathway. We therefore asked whether the Torso receptor is a druggable target in pest genomes. We developed a bimolecular luminescence complementation assay in insect cells to measure activation of Torso signalling by Trunk and its modulation in response to antagonists. The assay was used to screen 2500 compounds across four libraries and resulted in identification of compounds which effectively inhibited Torso signalling. A selection of these compounds tested in feeding experiments on Drosophila larvae significantly delayed the onset of pupariation compared to vehicle-treated controls, validating their efficacy in inhibiting Torso signalling in-vivo to delay the timing of moulting. We are currently testing the effects of their exposure on honeybee larvae. Our results validate a novel target in insect pest genomes and has identified chemistries with potential to formulate bee-friendly insecticides.

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O22-2

Enriched biocompost production from sugarcane bagasse using biotechnological processes

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The objective of the present study was to optimize fast production of enriched biocompost from sugarcane bagasse using native effective microorganisms at lab and pilot scales. To do this, a dynamic and controllable solid state bioreactor with 4 containers was designed for modeling and simulation of the composting process to isolate effective microorganisms. Four mesophilic and thermophilic bacterial strains with high cellulose, gluganase and amylase activities were selected and characterized. The lab scale compost production was performed using bagasse, filter cake and vinasses as base materials in all treatments (exception for control). The microbial cocktail (10^6–10^7 cells/g waste), chicken manure or urea were used as improvers. The treatments were as follows: T1 – chicken manure and microbial cocktail, T2 – chicken manure, T3 – urea and microbial cocktail, T4 – urea, and T5 – control (only bagasse). The results showed that the maximum temperature increase (60 °C) and the maximum C/N ratio (15.9) and EC reductions were observed for T1, followed by T2. The highest phosphorous (1.1%), potassium (1%), total nitrogen (2%) and nitrate (210 mg/kg) and also the lowest ammonia (67 mg/kg) contents were observed in T1. The fastest and maximum germination for cress (*Lepidium sativum*) was observed in T1 and T2, respectively. The wheat plants cultivated on the compost produced from T1 showed significantly higher height, and fresh and dry weights compared to other treatments. The results of pilot experiments confirmed those of lab scale experiments, and T1 showed high increase of temperature (up to 75 °C) and significant decrease of C/N ratio during the first month. Also, all physicochemical quality parameters, including nitrogen, phosphorous and potassium contents were significantly higher than that in the control.

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O22-3

Molecular upgrading of the performance limits of bacterial sensors for the remote detection of buried explosive devices

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Current methodologies for the detection of antipersonnel landmines still require the presence of personnel in proximity to active mines, with the obvious consequent risks involved. In an attempt to provide a solution, we have recently reported an *Escherichia coli*-based bioluminescent and fluorescent bioreporter system for the stand-off detection of buried trinitrotoluene (TNT)-based explosives. However, further enhancements to the biosensor's sensitivity were required to detect the lower concentration range of explosive vapors above buried landmines.

One of the approaches employed for this purpose was based on unravelling of the molecular metabolic networks that control the biotransformation of the 2,4-dinitrotoluene (DNT), the volatile impurity accompanying TNT. This has allowed us to identify trihydroxytoluene as the main inducing molecule of the *yqjF* gene promoter, the sensing element of the above-mentioned sensor strain; several genes involved in DNT metabolism were also identified, including *yhaK*, a deletion of which resulted in a 60-fold increase in the biosensor's response to DNT.

To further enhance the bioreporter's capabilities we have developed a high-throughput mutational screening assay, in which a plasmid containing the sensing-reporting elements was systematically transformed into a library containing all non-lethal gene deletions in *E. coli*. Upon screening of the transformed library with DNT we identified novel elements which were not formerly known to be related to DNT metabolism, and which displayed dramatic effects on the transcription rate of *yqjF* in response to DNT. We will show how the combination of these research approaches has led to the construction of significantly improved bacterial TNT-detecting biosensors.

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O22-4

Estimation of methane emission in organic paddy fields using remote sensing indexes

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This research studied methane emission in organic paddy fields in order to compare the correlation between methane emission and different vegetation indexes and to build a prediction equation for methane emission in organic paddy fields based on vegetation indexes and remote sensing data. The research site was in Thung Kula Ronghai area, Pathumrat District, Roiet Province, where organic *Hom Mali 105* variety is grown. The findings show that during seeding to tillering, CH_4 emission was in the range of 0.193–0.760 $mgCH_4\ m^{-2}\ h^{-1}$. Tillering to ripening, CH_4 emission was in the range of 1.666–2.446 $mgCH_4\ m^{-2}\ h^{-1}$. Ripening to harvesting, CH_4 emission was in the range of 0.935–2.273 $mgCH_4\ m^{-2}\ h^{-1}$.

Methane emission from organic rice fields was found to increase with the age and growth of rice stalks and to decrease at the period before harvesting. This finding is consistent with the vegetation indexes, which continue to increase along the rice growth stages until the maximum leaf growth when the vegetation index is at a high. The index then decreases slightly at the stage during seed growth and leaves turning yellow.

When taking the 4 types of vegetation indexes, namely, NDVI, GNDVI, SAVI and IPVI to calculate the Pearson coefficient correlations, it was found that GNDVI had the greatest correlation with methane emission from the rice paddy, i.e., 0.752. Then the predicting equation for methane emission was built from the GNDVI parameter: $Y = -11.24x^2 + 15.21x - 3$.

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O22-5

Assessing the microbiome dynamics in three photo-bioreactors established for coking wastewater treatment: An orchestration between microalgae and bacterial communities

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The investigation of microbial community structures is a significant way to understand biodegradation capacities in biological wastewater treatment processes. Photo-bioreactors A, B and C received real coking-wastewater as influent with COD 776 ± 56, 1229 ± 85 and 2033 ± 27 mg/l, respectively. In phase-1 phenol was added to the influent, while dichlorophenol was added in combination with phenol in phase-2. Treatment efficiency of algal-bacterial systems was biomonitored through bioassays (phytotoxicity, Artemia-toxicity, cytotoxicity, algal-bacterial ratio and settleability). COD, phenol and dichlorophenol concentrations were also monitored. All systems efficiently detoxified the influents in phase-1. In phase-2, Systems B and C failed to detoxify the influents. Illumina-sequencing generated 2119749 effective sequences of 16S-rRNA gene from 21 samples collected from different influents and effluents. The number of observed species was significantly lower in effluent samples than influent samples, as some taxa dominated and contributed to the systems performance. Significant difference in microbial diversity between influent and effluent samples was detected. Proteobacteria (78%), Firmicutes (12%), Bacteroidetes (5%) and Deferribacteres (2%) were the dominant phyla in influent samples. While in effluent samples Proteobacteria (68%) and Bacteroidetes (25%) dominated. Failure in treatment in systems B and C at phase-2 was accompanied with significant difference in the microbial diversity. Significant relative abundance of anaerobic bacteria from Deferribacteraceae and Peptococcaceae families in influent samples conformed to the nature of coking-wastewater. The co-culture of microalgae shifted the microbiome and promoted the activity of Chitinophagaceae, Pseudomonadaceae and Xanthomonadaceae families. These bacteria are known for their catabolic diversity that enables xenobiotic degradation. The superiority of algal-bacterial systems was confirmed as co-culture of microalgae eradicated pathogenic bacteria as Arcobacter and Legionella genera in treated effluent.

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O22-6

A biorefinery approach for the conversion of forest wastes into polyhydroxyalkanoates

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Polyhydroxyalkanoates (PHA) are bacterial intracellular carbon/energy reserves that can be produced by mixed microbial cultures (MMC) using several waste streams. These biopolymers present physical–chemical properties that make them suitable as biodegradable and biocompatible thermoplastics. PHA production may consist of two or three steps, having in common a microbial culture selection and a maximization PHA production step, when using substrates rich in short-chain organic acids (SCOA). For a three-step process, when a high sugar content is present in the initial substrate a pre-fermentation step is required to enrich the feed in SCOA. In this work, the valorisation of forest industry residues into PHA by MMC in a three-step process is proposed, using as substrate pinewood residues converted to pyrolysis bio-oil.

Considering the pre-fermentation step, acidogenic fermentation of bio-oil in continuous operation mode resulted in the production of lactic, butyric, propionic, and valeric acids. For the selection step, a sequencing batch reactor operated under aerobic dynamic feeding was enriched using unfermented bio-oil. Both hydroxybutyrate (HB) production yield (>0.10 Cmmol/Cmmol S) and the maximum HB content (4–6%/wt.) were in the average range for the selection processes. In order to test the selected culture response for PHA accumulation, three feed pulses of acetic acid (30 mM) were performed, being completely consumed during each pulse. A maximum of 46% HB content was obtained, although the culture did not reach its saturation. The coupling of both systems, using a stable SCOA stream from the anaerobic fermenter, should improve these results.

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O22-7

Bacterial exopolysaccharide: A smart biomaterial to address the heavy metal stress

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Metals fetch serious environmental pollution causing a threat to human health and ecosystem. The heavy metal accumulation due to anthropogenic activities results in toxicological manifestation. The increasing presence of heavy metals in the microbial habitat compels the microbes to develop the ability to tolerate or resist the presence of heavy metals. Exopolysaccharides (EPS) production is one of the strategies of microbes to fight against the metal stress. EPS are microbial biopolymer which produced under stress of harsh environment and nutrition conditions. On this framework, the heavy metal stress consequences on exopolysaccharide (EPS) producing agricultural isolate, *Pantoea agglomerans* was studied. *P. agglomerans* show tolerance and mucoid growth in the presence of heavy metals, i.e., mercury, copper, silver, arsenic, lead, chromium, and cadmium. EDX confirmed the metal accumulation and further, FTIR determined the functional groups involved in metal binding. The ICP-AES used to determine the cell-bound and intracellular metal accumulation. Metal deposition on cell surface has released more Ca^{2+}. The effect on bacterial morphology investigated with SEM and TEM revealed the sites of metal accumulation, as well as possible structural changes. Each heavy metal caused distinct difference and accumulated as cell bound EPS with some intracellular deposits. The metal stress caused a decrease in total protein content and increased total carbohydrate with a boost in EPS. The high EPS production in the metal presence might protect the bacterial cell from metal stress. Thus the performance of *P. agglomerans* under metal stress indicated a potential candidate for metal bioremediation.

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Symposium 23: Novozymes Integrated Bioprocessing Symposium part 2

O23-S

Molecular development for industrial biotechnology using data analysis, protein structures and small leaps of faith

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The increased ease with which virtual workflows can be estab-lished coupled with real-world automation advances is enabling new exciting possibilities in the development of enzymes for industrial biotechnology. Key workflows, such as protein stabil-ity engineering and expression strain construction, that once were wholly manual, can now to a large degree be replaced by automated processes that yield greater data coverage, larger through-put and higher reproducibility. However, to enable these new processes it is still important that we understand the characteristics of the molecules we are trying to optimize and produce. Thus, good assays, biophysical characterization data, and the understanding of the relevant industrial processes is as important as ever, and a lack of one or more of these is often a key obstacle to developing a successful product.

In this presentation I will describe the trends that are open-ing up new possibilities in industrial biotechnology, and discuss these in relation to recent examples from our molecular develop-ment pipeline to provide a perspective on the future of molecular industrial biotechnology.

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O23-1

Enhanced soluble expression in Escherichia coli and miniaturized fed batch fermentation screening platform

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Escherichia coli is the host system of choice for the production of therapeutic proteins that do not require translational modification (e.g. glycosylation) for their biological activity. The soluble expression of biopharmaceuticals, like antibody fragments, is challenging since only part of the total target protein is correctly folded strongly depending on the genetic setting of the host cell and the culturing conditions.

Today, screening for the best production conditions and strains is still mostly performed in shake flask cultivations. We observed that screening results from batch cultivations were not always predictable for industrial fed-batch fermentation processes. Batch cultures have low volumetric cell and product yields. Dissolved oxygen concentration, pH as well as specific growth rate are not controlled and overflow metabolism impacts process performance. At Boehringer Ingelheim, we established a toolbox consisting of various genetic elements for the production of properly folded target proteins in the Escherichia coli periplasm.

Next to different bacterial strains, the toolbox also comprises elements like various promoters, leader peptides, genomic integration of the target gene expression cassette and the co-synthesis of helper factors. For the antibody fragment FabZ genomic expression and co-production of a helper factor increased the soluble titers by up to 10-fold. Com-bination of toolbox elements yields a large number of different expression strains.

To enable high throughput screening, a fully automated and feedable minibioreactor system integrated into a liquid handling system was established. Cultivations are performed in 10 mL scale under fed-batch fermentation conditions. It offers new possibilities in high throughput strain selection for industrial fed batch fermen-tation processes and first experiments promise good predictability.

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O23-2

Scaling down further: Model-based scale-down studies in minibioreactors

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Inadequate mixing and the associated concentration gradients in large-scale microbial bioprocesses have significant impacts on both recombinant protein quality and cell physiology. The impact of these effects on bioprocess efficiency are studied in scale-down bioreactors in the laboratory, usually after a potential bioprocess performs poorly in large scale, thus initiating a second process development phase. It is therefore desirable that the response of strains to heterogeneous environments is tested at the screening phase, to forestall unforeseen responses upon process scale-up.

In this work, we combine mechanistic models with a high throughput minibioreactor system to form a scale-down platform for studying the response of strains to scale-up effects at the screening phase. The mechanistic model is used to simulate the anticipated heterogeneities in large-scale bioreactors, and these are implemented in the minibioreactors using robotic liquid handling stations. As a demonstration, the platform was used to study the impact of different frequencies and amplitudes of glucose and dissolved oxygen gradients on the misincorporation of non-canonical amino acids (ncBCAA) into recombinant pro-insulin produced in Escherichia coli, using 24 parallel fed-batch cultivations in 2mag bioREACTORS® . The results show that in cultivations where the cells were subjected to rapid model-derived gradients, there was a marked increase in the accumulation and misincorporation of ncBCAA into the recombinant product, which significantly undermines the product quality. Thus, the platform offers the opportunity to combine strain screening with scale-down studies to select the most robust strains for process scale-up.

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O23-3

Production under the AOX1 promoter in methanol utilization negative *Pichia pastoris*: An efficient expression system for less intensive fermentation

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The classical *Pichia pastoris* (syn *Komagataella* spp.) fed-batch process, which is used for driving protein expression under the control of the AOX1 promoter (P_{AOX1}) exhibits several drawbacks. The consumption of methanol is accompanied by high heat generation and oxygen demand leading to extensive need for aeration and cooling in up-scaled processes. All of these issues have promoted the search for alternative simple processes that still allow the high productivities of the P_{AOX1}-system.

The methanol utilization phenotype of *P. pastoris* is determined by the presence of its alcohol oxidase genes *AOX1* and *AOX2*. Knock-out of one or both *AOX* genes leads to methanol utilization slow (mut^S) and methanol utilization negative (mut^-) phenotypes, respectively. Ideally, the use of mut^- strains would circumvent or lessen the problems associated with methanol utilization while still retaining the inducibility and high expression of P_{AOX1}. Thus, we tested mut^- strains for secreted protein production under the control of P_{AOX1}. In this scenario a non-hazardous carbohydrate carbon source is used for biomass generation while methanol is used for induction of P_{AOX1}. Screening data as well as fed batch bioreactor cultivations show that the mut^- strains are capable of producing secreted recombinant proteins. Protein production is achieved when the methanol induction is parallel to or in succession of the carbohydrate feed. In the latter case, protein production does not coincide with an observable biomass increase. This represents a specific case of growth independent protein production in *P. pastoris*.

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O23-4

Alkaline protease production from newly isolated plant growth promoting rhizobacteria

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Plant growth promoting rhizobacteria (PGPR) are found in soils and they play an essential role in nutrient uptake of the plants. These bacteria are potential producers of enzymes such as proteases, glucanases and chitinases. Alkaline proteases are commonly used in industrial catalysis, hence the interest of finding new sources for produce these enzymes.

In this work, the potential protease activity of 10 new isolates of *Bacillus* genus, was studied, and then, after selecting the most promising strains, their proteolytic activity when grown in synthetic medium was measured.

A specific medium for protease production was then designed, using a Plackett–Burman design for the selection of the culture media ingredients and Surface Response Methodology (SRM) for the optimization of the final composition of the medium. In order to identify the medium's ingredients with the greatest influence on the proteolytic activity, a simplified Plackett-Burman design in 12 runs was used to evaluate the effect of 7 potential ingredients at two-level concentration. The optimal concentration of the two ingredients increasing more the proteolytic activity of the culture was obtained using a 3-level factorial experiment design.

The biosynthesis process for protease production was scaled-up to a 5 L stirred tank bioreactor and finally the recovery of the enzyme was done. In this final step, microfiltration and ultrafiltration equipment for removal of cell material and concentration of the crude enzyme extract were used and the purification of the alkaline protease was done using a fast protein liquid chromatography (FPLC) equipment.

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(such as ethane, propene and para-xylene). As a result bio-based polymers often contain greater residual chemical functionality in their chains, with groups such as alkenes and hydroxyls commonly observed. These functional groups can act as sites for post-polymerisation modification (PPM), thus further extending the range of applications for bio-based polymers by tailoring the polymers final properties [1].

In this work, *Candida antarctica* lipase B (CaLB) was used to catalyze the synthesis of functional aliphatic polyesters based on dimethyl itaconate (DMI) and various aliphatic diesters and diols. The use of enzyme for such polymerization reactions is of particular interest due to the undesired side reactions (such as isomerization, branching and crosslinking) encountered during the traditional metal-catalyzed processes [2]. Several aliphatic and functional polyesters such as poly(1,4-butylene adipate), poly(1,4-butylene itaconate) and poly(glycerol adipate) among others were successfully produced using a solventless polycondensation protocol. All polymers were characterized via ^{1}H and ^{13}C NMR spectroscopy, GPC, DSC and TGA.

This work sheds light on the possibility of synthesizing, via enzymatic catalysis, functional, clickable oligoesters that can be further crosslinked or coupled with end-chain moieties in a 2nd reaction step.

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H3-2

Grave-to-cradle: The potential of autotrophic bioprocesses in bioplastic production

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In 2015 the annual average concentration of atmospheric CO_2 rose for the first time above 400 ppm. This raise was clearly related to ever-growing industrial activity and combustion of petrol and its related products, for example, plastics, which annual global production is over 300 Mio tons. An interesting option to approach this problem is the biosynthesis of bioplastic from waste gases for the mitigation of the production of CO_2.

Here, biotechnology offers two basic approaches to use autotrophy for bioplastic production: photoautotrophic and chemoautotrophic growth. The first one is very attractive because the biopolymer production is inexpensive and only limited by available sunlight, nutrients and CO_2. The polymers produced are mainly polysaccharides (e.g. cellulose and starch), proteins (e.g. cyanophycin) and thermoplastic polyesters (e.g. poly(3-hydroxybutyrate) from Cyanobacteria). A typical representative for the chemoautotrophic polymer production is *Cupriavidus necator*. It produces PHB by metabolizing the gases CO_2, O_2 and H_2 under nitrogen limitation. However, these growth conditions are quite challenging in terms of safety, but an appropriate control of the fermentation using process analytical technology and additional safety precautions allow sensible risk management.

In our laboratory, we investigated the growth performance of *Rhodospirillum rubrum*. Under anaerobic conditions this strain is able to consume carbon monoxide (CO) from syngas, by using the water gas shift reaction catalyzed by the CO-dehydrogenase. CO_2 produced this way is partially assimilated into biomass and the formation of PHB, the accumulation of the latter being boosted by acetate addition.

Advantages and disadvantages of these approaches will be particularly addressed.

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H3-3

A quest for abundant and sustainable carbon sources in polyhydroxyalkanoates production – Are seaweeds the answer?

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Polyhydroxyalkanoates (PHAs) are bacterial polyesters that can replace synthetic plastics in numerous applications with the advantage of being biodegradable, though still not competitive due to production cost, mainly of C-source.

Because of their high carbohydrate content (25–60% DW) and absence of lignin, macroalgae are promising feedstocks for biobased chemicals and materials. In contrast to terrestrial crops, seaweed do not require land, fresh water and fertilizers. The major challenge concerning the complete utilization of macroalgal carbohydrates is their complex polysaccharide composition. Besides a glucan fraction (cellulose, starch or laminarin), seaweed possess polysaccharides that yield monomers not easily metabolized by most industrial strains.

The whole biomass of the green alga *Ulva lactuca* and residues of *Gelidium sesquipedale* were tested as raw materials for PHA production. After agar extraction, *Gelidium* leftovers contained non-negligible amounts (48%, w/w) of carbohydrates [1]. Acid pretreatment combined with enzymatic cocktails achieved 90% sugar recovery (glucose, xylose and rhamnose) in the case of *Ulva* and 97% (glucose and galactose) for *Gelidium* residues.

Algal hydrolysates with ca. 20 g/L total sugars were obtained and tested as C-source. A search enabled the selection of marine bacterial strains with considerable PHA accumulation ability (*Halomonas* sp.) and capable of consuming the main monosaccharides present. Bacterial growth and PHA production on the hydrolysates were assessed at shake flask scale and assays in 2L STRs are being performed.

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H3-4

Polyesterases: Design, function and application

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In recent years, hydrolases like cutinases, esterases and lipases have been recognized as powerful tools for hydrolysis of synthetic polymers such as polyethylene terephthalate (PET) as an environmentally friendly alternative for harmful chemical methods. Polyesterases have been used for the recycling and functionalization of polyesters for example to enhance hydrophilicity which in turn improves, for example, breathability, moisture uptake, antistatic behavior, and handle of textiles. Nevertheless, the activities of polyesterases on synthetic polyesters is rather low since they have been designed by nature for other substrates [1]. Hence, different strategies of enzyme engineering have been applied to improve

their activity especially considering sorption on hydrophobic surfaces for functionalization, processing and degradation of non-natural polymers [2]. These approaches involved, for example, enzyme surface engineering, incorporation of non-canonical amino acids and their synergistic combinations [3]. In addition, mechanistic studies were performed using sophisticated analytic tools such as Quartz crystal microbalance analysis for comprehensive analysis of the combined effect of polyester structure and enzyme active-site accessibility on enzymatic polyester hydrolysis [4].

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H4-1

Plant seed production of biopharmaceuticals: Bridging red and green biotechnology

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I will describe how we evaluated the opportunities provided by biopharmaceuticals produced in plant seeds in various collaborations, in this way covering green, red and white aspects of biotechnology.

We obtained proof of concept for the protection of piglets from gastric bacterial infection by a feed additive of crushed seeds expressing specific antibodies. Such a feed additive could for instance be used upon weaning of piglets when they are most sensitive for gastrointestinal infection and in this way reduce the therapeutic use of antibiotics. Also, seed-produced antibodies open the possibility to treat gastrointestinal infections in humans by oral consumption of a crude seed extract, formulated to contain an effective dose.

Another application explored was the production of monoclonal antibodies (MABs) and vaccines in a glyco-engineered seed matrix. Because most therapeutic MABs and vaccines are administered as injectables, an issue of concern is the plant-specific glycosylation they might contain, which can result in adverse and allergenic reactions over time. Therefore, the GlycoDelete technology was applied and shown to be working in plant seeds. Upon eliminating and introducing specific enzyme activities in the seeds, several glycoproteins accumulating in GlycoDelete seeds uniformly contain only one single sugar moiety. In this way it would be possible to stock pile neutralizing glycan-free antibodies specific for seasonal/epidemic/pandemic infectious diseases in seeds and only start processing and purifying those in case of an infection outbreak.

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H4-2

Yeast biomass formation from carbon dioxide by rational metabolic engineering of *Pichia pastoris*

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Industrial processes based on the exploitation of fossil resources accumulate carbon dioxide (CO_2) and contribute to the greenhouse effect. The naturally occurring fixation pathways fail to keep the balance of CO_2 concentrations at a sturdy level with increasing emissions of CO_2. Recent advances in genome editing technics now facilitate the engineering of a great variety of organisms. We exploited this new playground of genetic tools to trigger the heterotrophic organism *Pichia pastoris* towards autotrophic behavior.

In this work we show that a rational design of a synthetic Calvin cycle in the methylotrophic yeast *P. pastoris* allows CO_2 incorporation and robust growth. The engineering blueprint is based on a modularization of the central carbon metabolism of *P. pastoris*. The repertoire of peroxisomal enzymes is supplemented with enzymes from the pentose phosphate pathway and from glycolysis. RuBisCO, Earth's most abundant enzyme, is integrated to carry out the fixation reaction of CO_2 (carboxylation) besides the second key enzyme of the Calvin cycle PRK. This strategy turns the peroxisome into a synthetic CO_2 fixation compartment (CO_2 fixation module). The energetically intensive synthesis of phospho-sugars from CO_2 is driven by oxidation reactions providing reduction equivalents in the form of NADH (Energy module). The resulting *P. pastoris* strain is able to incorporate CO_2 fueled by the oxidation of a co-substrate. Furthermore, the incorporation of CO_2 was verified by ^{13}C labelling followed by elemental analysis – isotope ratio mass spectrometry (EA-IRMS). We are the first ones reporting the creation of functional synthetic hemiautotrophy in a eukaryotic organism featuring robust growth on CO_2 as the sole carbon source.

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H4-3

Making the strange more familiar: Influence of germinality content on mAb expression potentials and thermal stability properties

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The antibody expression potential of individual monoclonal antibodies (mAbs) can differ significantly, despite recombinant Chinese hamster ovary (CHO) cell lines are developed and cultivated under comparable conditions. We conclude that these expression differences might result from the intrinsic antibody structure and its interaction with cellular compartments of the folding and secretion machinery. To explore responsible factors we defined a population of four mature naturally occurring mAbs and designed a germline derived cognate mAb of each. We assigned each naturally occurring mAb to the corresponding designed variant composed of the nearest related germline genes. To compare interrelated mature and germline antibody pairs we express them either stably in a defined chromosomal environment in CHO cells or as single-chain Fv-Fc antibodies (scFv-Fc) transiently in HEK293 cells. This complementary strategy allows the comparison of selected CHO clones versus HEK293 transient transfection pools and whole IgG versus scFv-Fc antibodies. To develop stable IgG expressing CHO clones,

we inserted the transgenes using the recombinase-mediated cassette exchange (RMCE) system, which enables a targeted single integration. Although the antibodies differ only in their variable sequence, different expression potentials are observed in the IgG expressing CHO clones and the scFv-Fc transfection pools. In addition, we performed differential scanning calorimetry (DSC) measurements of the purified IgG and scFv-Fc antibodies. A significant increase in thermal stability of two germline variants compared to their associated mature antibody is measured. These methods help us to investigate the phenomenon of differential antibody expression levels and their correlation to thermostability properties.

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H4-4

Controlled encapsulation of T helper peptides into functionalised liposomes

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Protective vaccination against the human immunodeficiency virus (HIV) is acknowledged as a promising approach to contain the pandemic. Intrastructural help (ISH) is an immunological phenomenon that was previously shown to harness pre-existing immunity against a distinct antigen (e.g. HIV-1 GagPol) to modulate and improve the immune response towards a vaccine antigen (e.g. HIV-1 Env).

We hypothesize that the concept of ISH can be realised in individuals with immunity against hepatitis B virus (HBV). By the use of liposomes that present native-like trimeric HIV-1 antigens on their surface and additionally carry HBV-derived immunodominant T helper cell epitopes within their interior we aim to improve the immune response against the HIV-1 antigen. Here we elaborate protocols for the controlled encapsulation of a model T helper peptide into liposomes that incorporate functionalised lipids for subsequent protein bioconjugation. We also present analytical methods for the characterisation of this vaccine precursor.

Zeta potential and particle size analyses were used to study interactions between peptides and lipid membranes. Moreover, ultrafiltration and quantitative HPLC based approaches were used to identify conditions for improved and controlled peptide encapsulation. Modifying the model peptide by adding charged residues to its N-terminus and C-terminus was proven to be useful in enhancing the interactions and hence the encapsulation efficiency. These modifications were shown to have no negative effects on proper presentation via MHC-II molecules after uptake by dendritic cells *in vitro*.

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H6-1

Novel first-in-class protein–protein interaction inhibitor for NOTCH targeted therapy

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Cellestia is a privately owned clinical stage biopharmaceutical company located in Basel developing innovative first-in-class anticancer drugs originating from its in-house discovery platform. Cellestia's lead molecule is an anti-NOTCH agent and developed to address NOTCH driven human cancers. The NOTCH pathway is a key oncogenic cascade implicated in several human cancers leading to poor survival. Despite its well-established role in human cancers, there are no approved drugs available for patients suffering from NOTCH positive human cancers. In human tumors, the NOTCH pathway can be activated by various genetic lesions such over expression of ligands/receptors, GOF mutations in NOTCH receptors, including protein stabilizing mutations in the PEST domain of NOTCH, chromosomal translocations, or loss-of-function mutations in the E3 ubiquitin ligase FBXW7 and other negative regulators of the pathway (SPEN, NUMB). Activation of signalling due to above mentioned mechanisms can be addressed in part using blocking antibodies against NOTCH ligands/receptors or small molecule inhibitors of the gamma secretase enzyme (GSIs). However, in human tumors, where NOTCH signalling is constitutively activated due to chromosomal translocations in the NOTCH receptors (NOTCH1 and 2), none of the above-mentioned strategies will be effective. Moreover, due to on-target and off-target toxicities associated with blocking antibodies and GSIs, these anti-NOTCH agents failed to advance in clinical trials, although some of them showed signs of clinical efficacy. Cellestia Biotech aims to develop a potent anti-cancer and yet safer drugs for patients suffering with NOTCH positive cancers. Cellestia's lead molecule is currently being investigated in first-in-human trials.

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H7-1

BBI JU: Bridging the gap between research and market to develop a sustainable bio-based economy in Europe

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BBI JU, Brussels, Belgium

The Bio-based Industries Joint Undertaking (BBI JU) is a €3.7 billion public-private partnership (PPP) between the EU and the industry. It represents the largest industrial and economic cooperation endeavour financially ever undertaken in Europe in this field. Before the creation of BBI JU in 2014, Europe had specific problems linked to lack of development of bio-based industries and lack of investment in the necessary infrastructure that was needed. Eleni Zika, Head of Programme of BBI JU, will explain how, through its collaborative model, this EU initiative is bridging the gap between research and market, stimulating the research & innovation in Europe and integrating economic actors along the whole value chain. The BBI JU is an important pillar of Europe's bioeconomy strategy, playing a key role in creating markets for bio-based products and enabling a sustainable bio-based circular economy. By demonstrating the scaling up effect, BBI JU is helping to convince brand owners to engage creating new markets and products for consumers, and leading to far-reaching impact on the economy at both regional and international level.

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H7-2

The European Research Council (ERC) calls for proposals

A. Ferrari

ERC, Brussels, Belgium

Biotechnology is a broad and very interdisciplinary area of research that has experienced notable advances over the last decades.

This area of research is highly interdisciplinary because it com-bines disciplines such as molecular biology, genetic and chemical engineering, modelling, synthetic biology, systems and evolution-ary biology.

Certain focus of biotechnology makes it a very timely topic of research as it potentially touches closely on many aspects of our daily life such as drugs development, energy production, the cre-ation of artificial DNA and forms of life.

To date, the European Research Council (ERC) has been support-ing over 280 projects in the area of biotechnology with over 500 million EUR invested in this research area.

This presentation will cover ERC funding schemes with specific information about the ERC LS9 biotechnology panel.

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Posters

P1-1

Preparation of heparin oligosaccharides using immobilized heparinase

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Much of the biology and pharmacology of oligosaccharides of heparin and heparan sulfate (H/HS) remains unknown and unexploited to date. Although heparinase immobilization was developed several decades ago as a tool to de-heparinize blood, it was never exploited to prepare heparin/heparin sulphate oligosaccharides. In the present investigation, we showed that the heparinase immobilization is inherently capable of producing longer oligosaccharides. Heparinase 1 immobilized on CNBr-activated sepharose retained high efficiency of unfractionated heparin depolymerization over a wide range of pH (5–8), temperature (5–50 °C) and reusability more than 10 cycles. An immobilized heparinase bioreactor has been developed and found better for degradation of heparin into higher oligosaccharides compared to immobilized heparinase used in batch system (shake flask level) and with free heparinase. The optimum parameters to get larger oligosaccharides using immobilized bioreactor was observed at pH 5.5, temperature 15 °C and flow rate 800 μl/min. Most importantly, the immobilized enzyme was found to produce larger proportions of variably sulfated oligosaccharides longer than di- and tetrasaccharides as compared to heparinase 1 in the free form. Overall, this technology offers a simple and cost effective route to preparation of larger amounts of sequences that can be expected to bind and modulate protein function.

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P1-2

Production of keratinase by *Bacillus atrophaeus* BN2

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A new class of proteases known as “keratinases” have an advantage over normal proteases and have replaced them in many industrial applications, such as nitrogenous fertilizer production from keratinous waste, animal feed, leather industry, detergent additive application and biofuel production. Above all, one of the major hurdles of enzyme industrial applications (cost effective production) can be achieved by using keratinous waste biomass, such as use of chicken feathers as fermentation substrate. This low cost waste material serves dual purposes: to reduce the fermentation cost for enzyme production as well as reducing the environmental waste load.

Fifteen keratinolytic strains were isolated and selected from chicken feathers and poultry soil (Kabylia region) on solid feather meal medium, the test of keratinolytic activity on liquid medium allowed to select the isolate BN2 which is the most producing of keratinases (100 U/ml) after 48 h of fermentation. A morphological, biochemical and genotypic identification of the isolate BN2 were done, the 16S rDNA sequence results suggest that this isolate may be assigned as *Bacillus atrophaeus* strain BN2. The better production of keratinases was obtained when the strain grown at an optimum temperature of 40 °C, an optimum pH of 8, an optimal concentration of NaCl of 2%. The keratinase activity remained thermostable at 50 °C for 15 h and have an optimum temperature of 60 °C, it showed a high specificity towards feathers's keratin (117 U/ml) compared to azure keratin (48 U/ml).

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1871-6784/

P1-3

ART (ARduino-based pH Tracker), a new tool designed for characterization of a highly thermostable carbonic anhydrase

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Carbonic anhydrase (CA, EC 4.2.1.1) is ubiquitously present in many kinds of cell and has turnover numbers approaching 106/s. It facilitate CO_2 uptake by rapid conversion to bicarbonate in aqueous solution as equation: $CO_2 + H_2O \rightarrow HCO_3^- + H^+$, thus called as sequestration or bio-mineralization and used for carbon capture storage (CCS). A highly thermostable a-type CA, from bacterium *Sulfurihydrogenibium yellowstonense* with full-length gene of 753 bp (denoted as SyCA) was heterologous expressed in *E. coli* BL21(DE3) by pET28a at first. A new tool: ARduino-based pH Tracker (ART) determined Wilbur–Anderson unit (WAU) for CA activity was first applied. Arduino is an open-source electronics platform based on easy-to-use hardware and software. ART was built to monitor pH change every 0.5 s, transferred signal through Bluetooth to computer, and accomplished further calculation and analysis simultaneously. As a result, the activity of recombinant SyCA is 20,558 WAU/mg at pH 4. Free enzyme and whole cell had 37.5% and 79.9% residual activity after heating at 80 °C for 100 min. Metal effect showed that no activity lost in NH_4^+, K^+, Na^+, Mg^{2+} and Ca^{2+} while Co^{2+}, Cu^{2+} and Zn^{2+} highly inhibited activity at 5 mM. The k_{cat} and K_m of SyCA are 5.98×10^6 (s^{-1}) and 1.58×10^{-2} (M), respectively. Arduino-based pH tracker, as a powerful tool, accelerates analysis of CA and enzymatic kinetic study. The SyCA is one of the most heat-resistant carbonic anhydrase and will be a good candidate to increase its enzyme activity by direct evolution.

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P1-4

Purification and some properties of the thermostable peroxidase from thermophilic actinomycetes

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Thermophilic actinomycetes have the abilities to produce a variety of thermostable lignocellulolytic enzymes involved in the degradation of lignocellulose. The present study aims to purify the peroxidase from the thermophilic actinomycetes, *Thermobifida fusca* NTU22. Some properties of the enzyme are also investigated in this study. By using bagasse as carbon source, the activity of the peroxidase that simultaneously accumulated in the culture broth from a 500 mL Hinton flask after 48 h of cultivation at 50 °C were measured as 1.14 U/ml. The enzyme from *Thermobifida fusca* was purified 15-fold as measured by specific activity from culture broth by DEAE-Sepharose CL-6B and Sepharose CL-6B column chromatography. The overall yield of the purified enzyme was 2.2%. The purified enzyme gave an apparent single protein band on an SDS-PAGE. The molecular mass of purified enzyme as estimated by SDS-PAGE was found to be 70 kDa. The optimum temperature for the purified enzyme was 70 °C. About 90% of the original activity still remained at 70 °C for 4 h. The optimum temperature for the purified enzyme was 70 °C. About 90% of the original activity still remained at 70 °C for 4 h. The internal nucleotide sequences of the purified enzyme were investigated by digestion with trypsin and sequence analysis using LC–MS/MS. There were eight sequences detected. Comparing with the protein sequences in the NCBI database, the internal sequences have the highest homology with an enzyme from *T. fusca* TM51.

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P1-5

Construction of high-performance biocatalyst, modified D-stereospecific amidohydrolase from *Streptomyces* sp. for the synthesis of candidate molecule of insecticide

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A cyclic-dipeptide, cyclo (D-Pro–L-Arg) (c(DP-LR)) is known as a specific inhibitor of family 18 chitinases. It is also regarded as the lead compound for the development of antifungal reagents and insecticides. D-Amino-acid-containing peptides including c(DP-LR) have been produced through organic synthesis and the complicated reaction steps and the racemization of amino acids present difficulties for organic synthesis. Therefore, enzymatically-catalyzed peptide synthesis is recognized as an alternative method for chemical synthesis. D-Stereospecific amidohydrolase from *Streptomyces* sp 82F2 (DAH) belongs to family S12 serine peptidase, DAH, recognizes D-amino acyl derivatives as acyl donor and L-amino acids and their derivatives as acyl acceptor substrates and catalyzes amide bond formation by aminolysis reaction in accordance with hydrolysis activity to produce dipeptides with a DL-configuration. In this study, we attempted the synthesis of c(DP-LR) using the aminolysis function of DAH. For the investigation of c(DP-LR) production by DAH, D-Pro benzyl ester and L-Arg methyl ester were used as substrates. After 30 min reaction, two products, D-Pro-L-Arg-OMe and c(DP-LR) were detected. The concentration of D-Pro-L-Arg-OMe was decreased and that of c(DP-LR) was increased by prolonging the reaction time, resulting in a conversion ratio of greater than 65%. To enhance the productivity of c(DP-LR) by DAH, the structure of substrate binding site of DAH was modified by site-directed mutagenesis. We found that some of the mutations highly improved the reaction rate for c(DP-LR) production. Our study highlights the potential of construction of high-performance biocatalyst of DAH as a tool for biologically active dipeptide synthesis.

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P1-6

Construction of a cell-free synthetic pathway for the production of lactic acid from spent coffee grounds

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Coffee is the second largest traded commodity after petroleum but almost half of it ends up as waste in form of spent coffee grounds. Over 50% of the spent coffee grounds are composed of carbohydrates, with mannose representing the most abundant sugar. However, a holistic approach for its efficient utilisation has not been addressed yet. Cell-free synthetic biology is developing into a platform technology enabling a rapid construction of enzymatic pathways for the production of platform chemicals and pharmaceuticals from various substrates. Here, we designed a cell-free synthetic pathway for the utilisation of mannose derived from spent coffee grounds to produce the platform chemical lactic acid. The concept for the synthetic pathway is based on a putative mannose metabolic pathway from the thermoacidophilic archaeon *Thermoplasma acidophilum*. We were able to reconstruct this metabolic route by heterologous overexpression of mannose

dehydrogenase and mannonate dehydratase genes, and successfully convert mannose into 2-keto-3-deoxygluconate (2-KDG) in a one-pot enzymatic reaction. Further addition of two more enzymes resulted in a conversion of 2-KDG into lactic acid in a controlled and stereo-selective manner. Solid binding peptides (SBP) show great binding capacity towards a wide range of solid materials for the directed immobilisation of proteins and enzymes on solid supports. By co-expression of a silica-specific SBP, the pathway enzymes can be immobilised onto cheap silica-based supports like zeolite. This creates a range of reusable biocatalatyic modules, which can be rapidly assembled for future constructions of cell-free production pathways.

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P1-7

Improvement of 1,3-propanediol production in *Klebsiella* sp. by co-expressing *Vitreoscilla* hemoglobin and formate dehydrogenase genes

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1,3-Propanediol (1,3-PDO) is one of the important products used in chemical industry, in particular for polyesters production (e.g. polyethers and polyurethanes). In this study, with gene engineering technologies, the productivity of *Klebsiella* sp. is increased by expressing the gene of *Vitreoscilla* hemoglobin (VHb). And under the condition that NADH has been known to increase the productivity of *Klebsiella* sp. by about 10%, it is discovered that the expressing of the VHb can also increase of concentration of NADH by 2.45 times as much. And consequently the final production of 1,3-propanediol, short for 1,3-PDO, is increased by 2.78 times.

Owing to the amount of NADH in *Klebsiella* sp. being a critical factor in this study, the gene fdh of formate dehydrogenase is transferred into *Klebsiella* sp. to promote the transformation from NAD^+ into NADH. After 24 h of time, it is discovered that genes *vgb* and *fdh* working together can increase the molar ratio converted and the productivity of *Klebsiella* sp. by respectively 79.94% and 0.77 $g\,L^{-1}\,h^{-1}$, which is 10–12% higher than VHb working alone. It can thus be concluded that having genes vgb and fdh work together can upgrade the transformation and therefore increase the productivity of 1,3-PDO.

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P1-8

Bifunctional metal affinity adsorbents for concomitant purification and immobilization of enzymes

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The development of loofa sponge-based bifunctional metal affinity adsorbents for the concomitant purification and immobilization of enzymes was reported in this study. Loofa sponge, exhibiting high porosity and pore volume, is an ideal matrix for the development of eco-friendly, supermacroporous adsorbent for the adsorption of enzymes from unclarified cell homogenates. To increase the surface density of hydroxyl groups for activation, hydroxyethyl cellulose (HEC) was grafted to loofa sponge. The metal chelating and protein adsorption capacities of the metal affinity adsorbent thus prepared (loofa sponge-HEC-IMAC) was increased by 117% from 2520 ± 45 μmol/g to 5478 ± 77 μmol/g and 62% from 1.51 mg/g to 2.45 mg/g for the model enzyme, His-tagged trehalose synthase, respectively. Bifunctional metal affinity adsorbents (loofa sponge-HEC-IMAC-Epoxy) was prepared by reducing the level of iminodiacetic acid conjugation to the epichlorohydrin-activated HEC-grafted loofa sponge, giving a surface containing both metal chelating groups for the selective adsorption and epoxy groups for covalent immobilization. Upon optimization, a bifunctional adsorbent with an epoxy group concentration of 13% was identified. In a repeated batch operation study for the conversion of maltose to trehalose under the catalysis of the His-tagged trehalose synthase, the bifunctional adsorbent-based biocatalyst retained 96% of the catalytic activity after 20 cycles, significantly higher than the control (68%). The results obtained in this study indicate that the loofa sponged-based bifunctional adsorbents are promising matrices for the purification and immobilization of His-tagged enzymes, especially from unclarified cell homogenates.

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P1-9

Nanobiocatalysts for multi-enzymatic cascade reactions

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Multi-enzymatic cascade reactions, i.e., the integration of several biocatalytic transformations offer a wide range of opportunities and new paths to the synthesis of high added value products. Such systems could improve biocatalytic processes by saving time and reducing waste while could being self-sufficient in terms of co-factor requirements.

Enzyme immobilization onto solid supports is a commonly used strategy to improve the stability and reusability of multi-enzyme systems. However, in order to preserve the catalytic properties of all enzymes involved in the biocatalytic system, the co-immobilization strategy and the type of immobilization support require careful selection.

Magnetic nanoparticles have been employed as promising supports for enzyme anchoring, as they offer the great privilege of separation from reaction mixtures by just applying an external magnetic field. Therefore, these nanocarriers are able to facilitate the immobilization procedure and the overall handling process.

In the present work, we study the use of magnetic nanoparticles γ-Fe_2O_3 modified with 3-(aminopropyl)triethoxysilane (APTES), for the co-immobilization of three enzymes β-glucosidase, glucose oxidase and horseradish peroxidase leading to biocatalytic nanoconjugates able to conduct three-step cascade reactions. Various factors that affect the catalytic efficiency and operational stability of these biocatalytic nanoconjugates were studied.

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P1-10

A rational design to enhance the resistance of *Escherichia coli* phytase AppA to trypsin

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Escherichia coli phytase appA, which hydrolyzes phytate, has been widely applied as an important feed supplement, but its resistance to trypsin needs to be improved. Six putative solvent-accessible amino acid residues (K74, K75, K180, R181, K183, and K363), which could be easily attacked by trypsin, were selected to improve trypsin tolerance of *Escherichia coli* phytase appA. Inspection of the three-dimensional structure and computational design via hydrogen bond analysis and optimal site mutations of K74D/K75Q/K180N/R181N/K183S/K363N, which strengthened the hydrogen bonding, were performed to generate three mutants. Results showed that the most beneficial mutant appA-M6 had a specific activity of 3262 U/mg with molecular weight of approximately 52–55 kDa. Similar to appA-WT, the optimal pH (4.5) and temperature (60 °C) of appA-M6 were unchanged. Compared with appA-WT, appA-M6 showed a significant enhancement in resistance to trypsin and a 3.8 °C increase in melting temperature (T_m). We concluded that mutations in trypsin cleavage sites resulted in decreased enzyme flexibility due to the introduction of hydrogen bonds, increased the enzyme stability against proteolysis and thermal denaturation. The mutant appA-M6 generated in this study could be applied for the large-scale commercial production of phytase and thus could benefit the food and feed industry.

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P1-11

Molecular engineering of L-aspartate-α-decarboxylase for improved activity and catalytic stability

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β-Alanine is an important precursor for the production of food additives, pharmaceuticals, and nitrogen-containing chemicals. Compared with the conventional chemical routes for b-alanine production, the biocatalytic routes using L-aspartate-α-decarboxylase (ADC) are more attractive when energy and environment are concerned. However, ADC's poorly-understood properties and its inherent mechanism-based inactivation significantly limited the application of this enzyme. In this study, three genes encoding the ADC enzymes from *Escherichia coli*, *Corynebacterium glutamicum*, and *Bacillus subtilis* were overexpressed in *E. coli*. Their properties including specific activity, thermostability and mechanism-based inactivation were characterized. The ADC enzyme from *B. subtilis*, which had higher specific activity and thermostability than the others, was selected for further study. In order to improve its activity and relieve its mechanism-based inactivation by molecular engineering so as to improve its catalytic stability, a high-throughput fluorometric assay of β-alanine was developed. From a library of 4000 mutated enzymes, two variants with 18–22% higher specific activity and 29–64% higher catalytic stability were obtained. The best variant showed 50% higher β-alanine production than the wild type after 8 h of conversion of L-aspartate, showing great potential for industrial biocatalytic production of β-alanine.

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P1-12

Screening for new plant hydroxynitrile lyases

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Hydroxynitrile lyases (HNLs) catalyze the synthesis of chiral cyanohydrins, key building blocks for pharmaceuticals and agrochemicals [1]. This class of enzymes is very diverse when it comes to protein sequence, protein structure and catalytic sites [2]. Because of this reason, the discovery of truly new enzymes is a demanding task since there is no sequence identity between non-homologous isofunctional enzymes.

To identify new enzymes we performed a field trip to the iSimangaliso-Wetland-Park on the east coast in South Africa. This national park features an extraordinary and unique flora and offers perfect conditions for the detection of new HNLs in different plant species. The screening was performed by protein extraction and a subsequent Blue-Native PAGE [3]. The gel was followed by an assay to identify active enzymes in the gel as described by Krammer et al. [4] This screening method was applied on 40 different plants in various stages of development to increase the probability of a positive result. Five samples turned out to possess HNL activity and were subjected to follow up analysis in the laboratory at the University of the Witwatersrand in Johannesburg, where HNL activity was confirmed.

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P1-13

Effect of low concentration silanization agent and enzyme on the immobilization of lipase on a silica-based support material

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Enzymes are biocatalysts that accelerate a reaction rate thanks to their high activity, selectivity and specificity properties. The immobilization process can provide the ability to reuse with enzymes [1]. Also, the activity and the specificity of the enzyme can be increased. There are mainly techniques for immobilization: adsorption, covalent attachment, cross-linking and entrapment [2]. Support materials require for immobilization process. The silanization strategy is the most utilized surface modification method for support materials [3]. In this study, 3-aminopropyl trimethoxysilane (3-APTMS) was used to obtain the modified silica. The free *Candida antarctica* Lipase B was immobilized on activated rice husk ash by cross linked method. The rice husk ashes were used as a support material, due to their high amount of silica. The impact of low enzyme and silane concentration on activity and immobi-

lization yield was investigated. Efficiency of immobilized Lipase B was obtained by UV spectrophotometric method. In the light of the research, reduction of the silane concentration has led to a decrease in overall activity. The specific activity has been increased by the reduction of silane concentration. Immobilization yield and specific activity values were calculated by decreasing the enzyme concentration. As a result of this research, decrease in the enzyme concentration was resulted the increase in immobilization efficiency when compared with the literature.

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P1-14

Comparison of biochemical properties between original and newly identified oleate hydratases from *Stenotrophomonas maltophilia*

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Oleate hydratases (OhyAs) catalyze the conversion of unsaturated fatty acids to 10-hydroxy fatty acids. 10-Hydroxy fatty acids are used as precursors of lactones and dicarboxylic acids, which are important industrial compounds. The reported OhyA and a putative fatty acid hydratase in *Stenotrophomonas maltophilia* were identified by genomic analysis. The putative fatty acid hydratase was identified as an oleate hydratase (OhyA2) by determining its substrate specificity. The activity of OhyA2 as a holoenzyme was not affected on adding cofactors, whereas the original oleate hydratase (OhyA1) showed an increasing activity. Thus, all characterized OhyAs were classified as OhyA1 or OhyA2 based on the activities of holoenzymes on adding cofactors, were determined by the type of the fourth conserved amino acid of FAD-binding motif. Compared with *S. maltophilia* OhyA1, the OhyA2 showed higher hydration activity towards unsaturated fatty acids, including oleic acid, palmitoleic acid, linoleic acid, α-linolenic acid, and γ-linolenic acid. Moreover, the specific activity of *S. maltophilia* OhyA2 towards unsaturated fatty acids, with the exception of γ-linolenic acid, was the highest among all reported OhyAs.

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P1-15

Hyaluronic acid oligosaccharides are substrate for β-N-acetylhexosaminidase from *Streptomyces plicatus*

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Hexosaminidase from *Streptomyces plicatus* is an enzyme that catalyzes hydrolytic cleavage of N-acetylglucosamine from oligosaccharides. Its activity on substrates such as chitin oligosaccharides and glycans from glycoproteins are known. Here we describe activity of this enzyme towards hyaluronic acid oligosaccharides. Hyaluronic acid is a linear polysaccharide comprised of altering molecules of N-acetylglucosamine and glucuronic acid. We used HPLC for the analysis of the reaction products to show that the enzyme cleaves 1,4 bond between N-acetylglucosamine from the non-reducing end of an oligosaccharide and following glucuronide acid. Reaction conditions were optimized. The length of substrates varied from 4 up to 22 sugar moieties and all of them were accepted by the hexosaminidase, however, its activity decreased with increasing substrate length.

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P1-16

Optimization and enzymatic synthesis of rose aromatic ester (2-phenylethyl acetate) by lipase

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2-Phenylethyl acetate (2-PEAc) is a highly valued natural volatile ester with a rose-like odor that is widely used to add scent or flavour to cosmetics, soaps, foods, and drinks. Most of the 2-PEAc are now produced by chemical synthesis or extraction. However, chemical processes have several drawbacks, such as high/long reaction temperature/time, side products, discharging wastewater, irritating smell, corrosiveness of machine, etc. In this study, a continuous packed-bed bioreactor was used for synthesis of 2-PEAc by transesterification of ethyl acetate (EA) with 2-phenethyl ethanol (2-PE) catalyzed using Novozym® 435, 3-level-3-factor Box–Behnken design and response surface methodology (RSM) were employed to evaluate the effects of synthesis parameters, including concentration of 2-PE (100–500 mM), flow rate (1–5 mL/min) and reaction temperature (45–65 °C). Based on ridge maximum analysis, the optimal reaction conditions for synthesis of 2-PEAc were: concentration of 2-PE 62.07 mM, reaction temperature 54.03 °C, and flow rate 2.75 mL/min. The molar conversion of predicted values and actual experimental values were 100.22% and 99.01 ± 0.09%, respectively. In conclusion, the immobilized lipase was used and 15 reaction conditions were tested in order to find the combination for maximum yield. The optimization of 2-PEAc synthesis catalyzed by Novozym® 435 was successfully developed.

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P1-17

Effects of silanization reagent and enzyme concentrations on immobilized enzyme by activated with TMSP-DA

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In this study, rice husk as a result of rice production and free *Candida antarctica* lipase B (CALBL) were used. The outer layer of the husk collected from the rice production factories is quite rich in terms of carbon and silica content. The rice husk was burnt. The rice husk ash has at least 60% rich silica content. It is therefore economically suitable for the production of silica-based materials. High silica content rice husk is preferred for ash lipase immobilization. In this study, the burning of rice husks continued at 600 °C for 6 h. Subsequently, the surface of the rice husk crown was modified using 3-methoxy-silyl propyl ethylene diamine (TMSP-DA), a silanization reagent, to yield functional amine ($-NH_2$) groups on the surface. Free *C. antarctica* lipase B (CALBL) was immobilized by physical adsorption onto rice husk ash. In immobilized enzymes, different TMSP-DA (2%, 3%, 4%) and enzyme (0.2, 0.3, 0.4) concentrations were investigated. The effect of low silanization and enzymes

concentration on activity and immobilization efficiency was investigated. Immobilization and relative activity at low enzyme and silanization concentrations are much higher than at higher concentrations of enzyme and silanization. At high concentration, the activity is low because the multilayer binding state will occur so that the enzymes are closed to the active sites.

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P1-18

Kinetic resolution of 1-phenylethanamine in a solvent-free system by free and immobilized lipase

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In a solvent-free system, 1-phenylethanamine was resolved by a lipase-catalyzed acylation reaction. Vinyl acetate was regarded as the optimal acyl donor for the resolution of 1-phenylethanamine, after investigating the effects of different acylation reagents on the reaction. Higher resolution efficiency can be obtained in a solvent-free system when vinyl acetate acts as both the acyl donor and solvent, in contrast to reactions involving other organic solvents. LIP5 was screened as the optimal lipase for the resolution of 1-phenylethanamine. The optimal reaction conditions were as follows: 600 mmol/L substrate concentration, 70 mg LIP5, 2 mL vinyl acetate, 12.6 × *g* rotating speed, 35 °C, 8 h. The conversion, enantiomeric excess of (R)-N-(1-phenylethyl)acetamide, and *E* value reached 43.1%, 98.4%, and 275 respectively with free LIP5 as catalyst. The prepared Fe_3O_4 at chitosan nanoparticles were used for covalent immobilization of LIP5 by chemical conjugation. The optimal immobilization conditions were obtained as follows: enzyme concentration 2.4 mg/ml, pH 8.5, time 3 h and temperature 35 °C. Under these conditions, a high immobilization efficiency of 78% and the conversion of (R)-N-(1-phenylethyl)acetamide is 48.2%. The conversion, enantiomeric excess of (R)-N-(1-phenylethyl)acetamide, and *E* value reached 48.2%, 98.6%, and 463 respectively with immobilized LIP5 as catalyst. Stereoselectivity and resolution efficiency of immobilized LIP5 was higher than that of free LIP5.

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P1-19

New multilayer magnetic biocatalyst for esterification and transesterification reactions

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The immobilization of enzymes onto nanosized carriers attracted increasing scientific and practical interest, since the higher surface area can lead to increased enzyme loading and lower diffusional limitation. Particularly, magnetic nanoparticles are among the most promising supports, due to various functionalization possibilities and easy recovery, even form suspensions with higher viscosity. In this work, single-core magnetic nanoparticles with controlled size (in the range of 20–30 nm) were obtained by the co-precipitation method and were coated with an organic shell based on poly(benzofurane-co-arylacetic acid) or Nα,Nα-bis(carboxymethyl)-L-lysine hydrate, providing different (carboxyl, lactone, amino) reactive units. Lipase from *Candida antarctica* was immobilized on these magnetic nanoparticles by covalent binding, sol–gel entrapment and a combination of these methods, allowing the formation of a hybrid organic and silica layer for more efficient immobilization. Silane precursors holding glycidoxypropyl functional groups, like as 3-glycidoxypropyl-trimethoxysilane, (3-glycidoxypropyl)-bis-(trimethylsiloxy)methylsilane, and 1,3-bis(glycidoxypropyl)-tetramethyldisiloxane, have been employed, together with tetramethyl orthosilicate, for the synthesis of the functionalized silica layers. The morphological, structural and magnetic properties of the novel biocatalysts were assessed by TEM, SEM-EDX, X-ray photoelectron spectroscopy (XPS), vibrating sample magnetometry, while the biocatalytic activities were evaluated in esterification and transesterification reactions, particularly for the synthesis of aroma esters of natural carboxylic acids and for the kinetic resolution of specific secondary alcohols. Financial support from the Executive Unit for Financing Education Higher Research Development and Innovation (UEFISCDI), Project PN-III-P2-2.1-PED-2016-0168 is gratefully acknowledged.

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P1-20

Enhancement of the enzymatic saccharification of cellulosic materials using synthetic cocktails of the engineered fungal cellulases

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Cellulose is the most abundant organic substance on the Earth. The monomer unit of cellulose, glucose, is a starting point in biotechnological production of different value-added products, such as organic acids and alcohols, diols, alkanes, alkenes, biomaterials and biofuels. Attempts of scientists are aimed at increasing the efficiency of the enzymatic transformation of cellulose to glucose catalyzed by a cellulase multienzyme system including cellobiohydrolases (CBH) and endo-1,4-beta-glucanases (EG) as key enzymes.

Penicillium verruculosum is an effective fungal producer of the highly-active extracellular cellulase complex, in which CBH I (Cel7A), CBH II (Cel6A) and EG II (Cel5A) are the major components, comprising up to 70% of the total protein content in the culture broth. Previously, we have obtained the recombinant mutant forms of these enzymes with enhanced cellulase activities (up to 35%) through bioengineering of the enzyme N-glycosylation sites. In this study, we used the engineered forms of EG II, CBH I and CBH II as components of the binary and ternary enzyme mixes in hydrolysis of Avicel and a pretreated lignocellulosic substrate. In all the experiments, purified beta-glucosidase was extra added to the reaction system to provide the complete conversion of oligosaccharides to glucose. Using the synthetic cocktails of the engineered cellulases provided a significant increase in the glucose formation from cellulosic materials (up to 40%, depending on the mix composition and time of hydrolysis) compared to the cocktails of the wild-type enzymes of the same composition.

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P1-21

A tandem bio-chemo catalytic approach to the sustainable production of malic acid from glucose

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Petroleum-derived products are used in industry as platform chemicals for the production of high value commodities, including paints and specialist coatings. As highlighted by a 2004 US Department of Energy report, malic acid is a platform chemical in high global demand alongside other four carbon diacids. Fossil fuels are a finite source and their limited supply demands the development of renewable alternatives to petrochemically-derived products. Bio-based production is heavily dependent on microbial fermentation processes, which are restricted by the physiological limits of the cellular production system. A key challenge is the requirement to control a cell's multiple metabolic pathways that lead to formation of unwanted intermediates, thereby reducing conversion efficiencies. One solution to overcome this challenge is to use purified enzymes in a cell-free system. The aim of this project is to design an artificial enzymatic cascade to convert glucose derived from waste products into malic acid. The use of thermophilic enzymes allows for one step preparation of purified enzymes via heat denaturation. Immobilization of the enzymes provides the opportunity to maximise their activities. We have designed a 5 enzyme cascade that is cofactor balanced (NAD+, NADH) to enable the sustainable production of malic acid from glucose in a single reaction.

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P1-22

Bioproduction of Δ1-piperideine using engineered *Escherichia coli* strains

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Although the bioproduction of complex, functionalized *N*-heterocycles has been reported, bioproduction of unsubstituted platform *N*-heterocycles has not yet been achieved. Therefore, the suitability of putrescine oxidase from *Rhodococcus erythropolis* (PuO_{Rh}) for bioproduction of Δ^1-piperideine was studied. PuO_{Rh} catalyzes the oxidation of cadaverine to 5-aminopentanal. Although this product is known to cyclise spontaneously into Δ^1-piperideine, direct formation of this product catalyzed by PuO_{Rh} has not been demonstrated, except by using o-aminobenzaldehyde as a reagent to trap Δ^1-piperideine and shift the equilibrium for cyclisation. The PuO_{Rh} gene was cloned and expressed in *E. coli* BL21 (DE3) using the pET20b vector, and the His-tagged enzyme was purified. Steady-state kinetics of PuO_{Rh} were determined by monitoring oxygen consumption; the K_M and k_{cat} values were 0.24 ± 0.05 mM and $26.6 \pm 0.08\ s^{-1}$ for cadaverine, and 0.17 ± 0.03 mM and $147.4 \pm 0.4\ s^{-1}$ for putrescine, respectively. Whereas the k_{cat}/K_M values are lower than those reported using peroxidase-coupled assays, this result should represent the true kinetics of PuO_{Rh}. Using the purified enzyme, the conversion of cadaverine to Δ^1-piperideine was demonstrated qualitatively using LC–ESI-MS and ^{1}H NMR; 5-aminopentanal could not be detected. Δ^1-Piperideine also formed the corresponding dimer and trimers, a known spontaneous reaction, and the product ratio could be adjusted by varying the pH. This preliminary study indicates that PuO_{Rh} is suitable for bioproduction of Δ^1-piperideine. The next steps are to optimize the reaction conditions, quantify, extract and purify the products, and develop whole cell bioproduction of *N*-heterocycles from renewable feedstocks.

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P1-23

Polymerization of titania by silica-polymerizing enzymes

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Silica-polymerizing enzyme called silicatein found in the glass skeleton of sponges can catalyze the polymerization of silica under mild condition, i.e., at room temperature and neutral pH. It is known that composite filament of silicatein isoforms silicatein-α, silicatein-β, and scaffold protein silintaphin, catalyzes the polymerization of metal oxide such as titania under mild condition. Novel functional material composed of titania and biomolecules would be fabricated using attractive property of silicatein. However, silicatein-α and silicatein-β are easily self-assembled to form aggregation in aqueous solution. We modified silicatein-α and silicatein-β with soluble small protein to improve the solubility. These fusion proteins can be expressed in *E. coli* and are found to be stably soluble after refolding. The polymerization of titania by mixture of silicatein-α, silicatein-β, and silintaphin has been successfully achieved.

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P1-24

Methylation of intermediate product mediated the xantholipin biosynthetic pathway

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Methylation is a ubiquitous chemical modification of biological molecules in nature and modulates diverse biological processes. The methylation-dependent programming role has been found in the biosynthetic assembly line of several natural products. However, further more specific examples are needed for the recognition of its chemical checkpoint role in mediating the preassigned biosynthetic logic. Three methyltransferases were encoded in the gene cluster while only two methyl groups exit in xantholipin. As the methyltransferase XanM3 has been proved to catalyse the remethylation following a demethoxylation-dependent xanthone ring construction, all of the three methyltransferases seem to be indispensable for xantholipin biosynthesis based on previous genetic studies. In this work, we demonstrated that methyl groups introduced by methyltransferase at specific biosynthesis point play vital role for the occurrence of the subsequent modifications, and thus guaranteed the smooth biosynthesis of xantholipin. These findings help sharping the proposal that methylation can serve as important chemical mark for the rigorous biosynthetic progress.

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P1-25

Purification, characterization and kinetic properties of alpha-naphthyl acetate esterase from wheat flour (*Triticum aestivum*)

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Plant-esterase from wheat flour was purified via aqueous two-phase system (ATPS). The enzyme crude extract was first filtered through a polyethersulfone Omega™ ultrafiltration membrane, followed by two-step ATPS with PEG1000, NaH_2PO_4 and $(NH_4)_2SO_4$. Based on previous studies, the first ATPS consisting of 27.0% PEG1000 and 13.0% NaH_2PO_4 (w/w) was performed at room temperature and pH 5.0. The top phase was carefully separated, and 6.0% $(NH_4)_2SO_4$ (w/w) was added to form the second phase system under similar conditions, to obtain the pure enzyme that partitioned in the bottom salt phase. The purification thus obtained was 10.35 fold with an enzyme yield of 72.90%. Sodium dodecylsulphate polyacrymamide gel electrophoresis (SDS-PAGE) was used to estimate the molecular weight of the target esterase to be around 68 kilo Daltons (kDa). Using a 16 mM solution of alpha naphthyl acetate as substrate, the maximum activity of the purified enzyme, alpha naphthyl acetate esterase (ANAE) was found to be 0.78 U at the optimum conditions of 40 °C and pH 8.0 for 15 min of incubation. The Michaelis–Menten parameters K_m and V_{max} of the purified enzyme were estimated using Langmuir linear plot as 22.5 mM and 4.71 U mg^{-1}, respectively. Further purification was obtained by dialyzing the bottom ANAE rich phase in a solution of 0.04 M phosphate buffer with pH 6.5 to remove the salt, which was then concentrated using centrifugal tubes with Omega™ membrane. The highly pure ANAE so obtained was immobilized on the multiwalled carbon nanotube for the development of inhibition based enzyme biosensors for pesticides detection.

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P1-26

Trametes elegans: a fungal endophytic isolate from *Otoba gracilipes* as biocatalyst for natural flavors production

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Flavoring production by biotechnological processes has recently been a target for academic and industrial research, in which Fungi, that produces volatile flavor compounds by microbial methods and biotransformation, have been recognized as excellent candidates. One of them belongs to the Phylum Basidiomycete, which grows in decaying wood but few times reported as endophyte.

In this work, a fungal isolate from the cortex of the medicinal plant *Otoba gracilipes* (dry tropical forest, Colombia), was identified by ITS sequence analysis as *Trametes elegans*. This plant-associated microorganism was evaluated as a pleasant flavor resource by cultivating it on potato/dextrose broth and pulp of chontaduro (*Bactris gasipaes*)/dextrose broth at pH's of 3.5 and 6.0, for 30 days. After this time, the exhausted media were separated by filtration and recovered to vacuum-rotary evaporation at 40 °C. The volatile compounds produced as secondary metabolites were collected and tested for aroma profile. It was found floral and citric aromas.

On the other hand, to verify the ability of this strain as biocatalyst, fungal biomass from different growth phases was tested for LOX activity. Enzyme activity was determined spectrophotometrically by monitoring the increase in the absorbance at 234 nm due to the transformation of linoleic acid to the respective conjugated hydroperoxydiene.

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P1-27

Withdrawn

P1-28

Expression of recombinant alcohol dehydrogenase with attached glycans in *Escherichia coli*

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Enzyme immobilization represents one of the techniques offering higher reliability and reproducibility of industrial processes through increased enzyme stability, reusability and decreased energy demand. Immobilization techniques often require enzymes to be exposed to harsh conditions and result in decreased or even loss of the catalytic activity of the enzyme. One of the milder approaches in enzyme immobilization utilizes attached glycans as linkers to desired carrier which allow more flexibility in terms of subunit stability or co-factor binding. In this work, we have focused on construction of *Escherichia coli* based expression system capable of utilizing bacterial N-glycosylation machinery to deliver glyco-sylated recombinant yeast alcohol dehydrogenase further used in biotransformation of aromatic compounds used in food and cos-metic industry.

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P1-29

Application of ionic liquids in the enzymatic production of L-methionine

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The low solubility of L-methionine is the major limitation of L-methionine production in aqueous buffer. In this study, various ionic liquids (ILs) were investigated as additives in the enzyme catalyzed production of L-methionine. Among tested ILs, tetraalkylammonium hydroxide ILs showed enhancing effects not only on the solubility of L-methionine but also on the activity of enzyme (O-acetylhomoserine aminocarboxypropyltransferase). With tetraalkylammonium hydroxide, the solubility of methionine decreased whilst enzyme activity and stability increased with increasing alkyl chain length. In addition, the L-methionine solubility steadily increased as ILs concentration increased. L-methionine could be dissolved up to 232 g/L in 10% tetramethylammonium hydroxide solution. However, enzyme activity reached its highest activity when ILs concentration was 2.5% (3 times higher than that without ILs) and significantly decreased as ILs concentration further increased. The enzyme stabilities rapidly decreased after 2 h of incubation at enzymatic reaction conditions either with or without ILs. At the optimal concentration of tetraethylammonium hydroxide (2.5%), 74.0 g/L of L-methionine could be produced whereas 35 g/L of L-methionine could be obtained in reaction system without ILs.

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P1-30

Synthesis of polyvalerolactone via enzymatic ring opening polymerization in different reaction media

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The nature of employed solvents plays a crucial role in determining the stability of the biocatalyst and in the partitioning of substrates and products between the solvent and the biocatalyst in non-aqueous biocatalytic systems. In the current study, δ-valerolactone ring opening polymerization was carried out in different reaction media such as hexane, toluene and without solvent with both commercial enzyme, Novozyme 435 and with Candida antarctica lipase B (CALB) immobilized onto rise hush ash (RHA) via physical adsorption. For the determination of optimum reaction medium for enzymatic poly(δ-valerolactone) (PVL) synthesis, polymerizations were performed at various reaction periods (6, 24, 48, 72, and 120 h) and reaction temperatures (30, 40, 60, and 80 °C) for each reaction media. Molecular weight distributions and chain structures of the polymer samples were compared by gel permeation chromatography (GPC). The highest molecular weight (Mn = 9200 g/mol) was obtained at 40 °C at the end of 24 h in hexane medium via Novozyme 435. On the other hand, optimum reaction medium was toluene for the immobilized CALB. The highest molecular weight was reached as 8020 g/mol at 80 °C after 120 h.

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P2-1

Structure and function of a novel chitin-uptake channel by marine *Vibrio* species

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Marine Vibrios are chitinolytic bacteria that utilize chitin as a sole source of energy, but the chitin-uptake mechanism by these bacteria is not yet understood. Here, we describe the structural basis of chitin translocation through a chitooligosaccharide-specific porin, so-called chitoporin or VhChiP. The crystal structures show that VhChiP consists of typical barrel-like folds like other porins. The crystal structures of VhChiP in complex the N-acetamido groups of the fully-stretching sugar chain interacts intensively with charged residues inside the channel lumen, while the sugar rings stack against the aromatic side chains by hydrophobic interactions. Most strikingly, the N-terminal segment consisted of nine amino acid residues is found to locate on the periplasmic side and it most likely acts as a mechanical gate that controls the rate of sugar entry. Our structural and electrophysiological data provide the first model that elucidates a stringently regulated system for the highly efficient uptake of chitin nutrients by the marine *Vibrio* species.

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The International Space Station is an extraordinary microgravity laboratory and creates a research environment where processes can be observed without the distortions experienced on Earth.

Biotechnology R&D can benefit significantly from experimenting in space through the deeper understanding of biological systems which are affected by microgravity through a vast array of changes such as: global alterations in genes' expression, molecular signaling networks change, cell metabolisms modification, cell growth acceleration, tissues regeneration rates decrease, microorganisms virulence increase, 3-dimensional aggregation of cells into tissue-like architecture.

Space access is no longer restricted to agencies and governments only, but a new commercial spaceflight era has started. Based on a partnership with the European Space Agency, the International Commercial Experiment Cubes (ICE Cubes) service has been established as first such service in Europe to provide fast, simple and affordable access to space for research and technology.

Scientists and industries are more and more searching for answers in space and obtain results that push back the frontiers of understanding on Earth. New biotechnology discoveries can be achieved in space through the ICE Cubes service, transforming basic research findings into practical applications, such as new medicines or therapies, new (bio)-materials as well as innovative environmental, agricultural and industrial processes, technologies and products.

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P2-4

Analysis of the major risk factors and genetic variations of bone formation signaling pathways in patients of osteoarthritis

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Osteoarthritis (OA), the most common human arthritis, is considered as polygenic disease causing pain and disability in affected individuals, with very high incidence and prevalence across the globe. Several risk factors have been involved in OA development including age, gender, obesity, injury, family history and genetic variations. More than 80% of the adults with OA have knee OA (KOA) in which body weight plays a very crucial role. Moreover, genetic studies revealed that OA has a substantial hereditary component and the associated genes tend to be related to the process of synovial joint development. Therefore, the study was designed to identify the potential risk factors which include vulnerable age group, gender and genetic alterations in the development of OA. To execute the case control study, detailed history with clinical and radiographic examinations were recorded in patients' data collection form. Blood samples were collected with equal number of patients ($n = 400$) and controls ($n = 400$) having the age range from 40 to 60 years. T-ARMS PCR was used for genotypic analyses. It was observed that KOA constituted a major disease burden in patients, especially in females. Obese and overweight individuals were at high risk of developing the disease. A SNP (T>C; rs1044122) of a member from disintegrin and metalloprotease (ADAM12) gene representing the synonymous polymorphism (Ala824Ala) showed significant association with OA in local population. It can be suggested that the genetic variant (rs1044122) of ADAM12 gene play an important role in the progression of OA.

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P2-5

Antioxidant activity of crude extracts obtained from endophytic fungi isolated from *Otoba gracilipes* of dry tropical forest in Colombia

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Biodiversity of endophytic fungi associated with the medicinal plant *Otoba gracilipes* of dry tropical forest was determined and evaluated for the antioxidant activity of their crude extracts. A total of five Endophytic fungi were isolated from leaves and stem of *O. gracilipes*: EBB-ET01, EBB-ET11, EBB-ET12, EBB-ET13, and EBB-ET14. These were identified by ITS sequence analysis respectively as *Fusarium oxysporum*, *Colletotrichum* spp, *Schizophyllum commune*, *Annulohypoxylon stygium* and *Pestalotiopsis microspora*. Some of these species have been reported as producers of potential biopharmaceuticals because of their antioxidant properties and antitumor effects, among others as well as producer of extracellular polysaccharides (EPS). For this reason, the isolates were bioprospected for their ability to produce EPS's with antioxidant activity.

The fungal isolates were inoculated in liquid media (Potato Dextrose Broth) for duplicate and 30 days post cultivation they were centrifuged to recover the exhausted medium, which contained the extracellular polysaccharides. The crude extracts were obtained after a continued recovery and purification steps until to get it lyophilized. A solution of these extracts (0.2 g/mL) were evaluated for their ability to inactivate free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The crude extracts showed a percentage of inhibition DPPH higher than 50% after 5 min. These results suggest a high potential of these natural crude extracts as an antioxidant. These fungal isolates could be cultivated by using bioreactor to optimize the synthesis of polysaccharides with bioactivity.

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P2-6

Vibrio natriegens – an effective tool for protein production

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One of the first steps considered in the designing of the project where heterologous expression of proteins is needed, is the choice of the host organism. Among the currently available and most extensively studied expression microorganisms, *E. coli* remains the host of choice with its undoubted advantages from which the one of the most important is the short generation time. Recently, however, a bacterial strain, *Vibrio natriegens*, has been "rediscovered". This highly promising bacterium shows except the extraordinarily rapid growth rate also the ability to utilize sucrose and starch and the putative secretion of proteins into the media which could potentially facilitate the downstream steps in the whole process of protein production. However, no tailored expression vector plasmids have been constructed so far to fully exploit the potential of *V. natriegens* for laboratory practice and high-level protein production. In this work we examined the potential of *V. natriegens* to be used as a recombinant protein production system in relation to environmental challenges.

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P2-7

Withdrawn

P2-8

Genomic analysis of *Rhodococcus* sp. BH4 reveals two genes encoding different types of AHL-lactonase for quorum quenching

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N-acyl-homoserine lactones (AHL)-mediated quorum sensing (QS) plays a key role in biofilm formation. Therefore, the interference of QS, referred as quorum quenching (QQ), has been receiving a great deal of attention. *Rhodococcus* sp. BH4 showed not only the AHL-degrading activity, but also efficiently inhibited the biofilm formation. Despite the reputation of *Rhodococcus* sp. for possessing diverse QQ-enzymes, only an AHL-lactonase gene (*qsdA*) has been found. Recently, we found an additional gene for a putative QQ enzyme from sequenced genome of *Rhodococcus* sp. BH4 besides *qsdA*. The putative QQ enzyme of *Rhodococcus* sp. BH4 showed 46% and 45% amino acid sequence identities with AHL-lactonase and AHL-acylase (AidH and AiiO) from *Ochrobactrum* sp. T63 and *Ochrobactrum* sp. A44, respectively. We expressed the putative QQ gene (*jydB*) in *E. coli*, and the recombinant *E. coli* showed AHL-degrading activity. To determine whether this QQ gene encodes AHL-lactonase or AHL-acylase, we examined whether AHL gets restored by acid after the enzyme reaction. This AHL restoration experiment revealed that *jydB* gene from BH4 encodes an AHL lactonase. The deduced amino acid sequence of the BH4 AHL-lactonase revealed that it shares the known characteristics of α/β hydrolases fold family. In this study, we have identified the presence of different type of AHL-lactonase besides QsdA in *Rhodococcus* sp. BH4. High AHL-degrading activity and prominent biofilm inhibition capacity of *Rhodococcus* sp. BH4 compared to other QQ strains may be due to the presence of multiple QQ enzymes including two types of AHL-lactonases aside from the AHL-acylase and oxidoreductase.

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P2-9

New perspective of agricultural byproduct extract: *In vitro* and *in vivo* antioxidant and anti-cancer effect

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Skin cancer is largely divided into three types, melanoma, basal cell, and squamous cell carcinoma. Past few decades, skin cancer patient has been increasing steadily due to an increase of irradiation with rapid changes in global environmental and melanoma has accounted for the majority of skin cancer deaths. Moreover, people generally known that melanoma comes from melanocytes in the outer skin. But melanoma can occur every melanocyte in our body, such as intestines, eyes. Therefore, development of new melanoma treatment is very important. In this study, we focused on the anti-cancer effect of agricultural byproduct on B16-F10 melanoma. It has been reported that byproducts have a similar or even better effect than commonly used sites. In this study, we confirmed the anti-proliferation, anti-viability, antioxidant, and cell death induction effect of agricultural byproduct extract on B16-F10 melanoma cells *in vitro*. In addition, we measured the upregulation of cell death marker protein (cleaved caspase 9) and cell cycle arresting protein (p21). Furthermore, we observed the anti-metastasis and anti-cancer effect in the melanoma induced mice. As a result, we observed the anti-cancer effect *in vivo*. All of the results show that agricultural byproduct has an anti-melanoma effect both *in vitro* and *in vivo*. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01323005)" Rural Development Administration, Republic of Korea.

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P2-10

Graptopetalum species extracts down-regulate proliferation through an anti-oxidation effect on skin cancer cells

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Cancer is still a life-threatening disease in modern society. Among many cancers, skin cancer is growing steadily. Therefore,

we investigated the efficacy of natural plant extract in skin cancer cell, which occurs in many people. Normal cells are quite regulated by various factors in proliferation or growth; however, Cancer cells require a bunch of energy to proliferate themselves and during the period. In addition, the higher reactive oxygen species (ROS) levels are important to many cancer cells, accordingly, we confirmed the natural plant *Graptopetalum* extracts inhibitory effect on skin cancer cell. The current treatment radiation or chemotherapy is using for cancer therapy for a long time, but these kinds of therapies have a difficult point that the condition of the patient and various side effects. Therefore, *Graptopetalum* a natural plant extract, have anti-proliferation and anti-viability effect on skin cancer cells through antioxidant effect and expected that can lower the side effects. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01323005)" Rural Development Administration, Republic of Korea.

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P2-11

Upregulation of cell survival rate and adipogenesis by succulent plant extract on porcine cells via antioxidant effect and fatty acid synthase expression

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Meat consumption is rise due to global population as well as income growth. Livestock industries are actively studying functional feed additives to increase the productivity of high quality meat. Despite this investment, feed additive that can increase the productivity of high-quality meat cannot be developed yet. Therefore, it is necessary to develop a feed additive that can improve the productivity of high quality meat by controlling the immunity and lipid synthesis of livestock. Succulent plants, also known as cacti, have been mainly used for ornamental purposes, and there is little known about the efficacy except for a few species. In this study, we confirmed the effect of succulent plant extract on oxidative stress, cell death, and lipid accumulation in the porcine cells. We confirmed the downregulation of intracellular reactive oxygen species (ROS) levels through flow cytometry analysis. Also, upregulated the cell proliferation and inhibited the cell death. In addition, lipid accumulation was upregulated with succulent plant extract treatment. All of the results show that this succulent plant extract has potentials for porcine feed additives with immune cell death inhibition and lipid accumulation induction effect. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01323005)" Rural Development Administration, Republic of Korea.

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P2-12

Development of ultra-fast detection method for GMOs

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In this study, we tried to develop a method to examine genetically modified (GM) rice, potato and papaya more accurately and quickly.

5 events of GM rice (Bt63, KMD1, Kefeng6, Kefeng8, LLRice62), 3 events of GM papaya (55-1, 16-0-1&17-0-5, PRSV-HN), and 5 events of GM potato (SPS-E12, SPS-F10, SPS-J3, AM04-1020, EH92-527-1) were used for developing ultra-fast detection method.

In order to develop a rapid detection method for GM rice, GM papaya and GM potato, the optimal conditions were derived using ultra-fast polymerase chain reaction and the specificity and sensitivity were verified.

As a result, all methods were specific for cross-reactivity to other crops, 42 GM events, with sensitivities ranging from 0.04% to 0.8%.

These methods are expected to be used more quickly and precisely in the field test.

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P2-13

Isolation of cellulase-producing *Cellulomonas* sp. from wastewater sludge for solubilization of primary sludge

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The activated sludge process is one of the most widely used methods for biological wastewater treatment plants. The excess sludge could be converted to biogas energy by anaerobic digestion process, but it still contains non-degradable organic materials such as protein, lipid, and cellulose. Therefore, solubilization of the non-degradable organic materials in the primary sludge is essential for effective production of biogas. Cellulase is commonly used in several agricultural, industrial and sludge treatment processes. From the course of screening for useful enzyme-producing microorganisms, we isolated cellulase-producing strains from the sludge of wastewater treatment plants in Korea and their cellulase activity was tested. On the basis of 16S rDNA sequencing, morphological, and biochemical studies, a new isolate was identified as a *Cellulomonas flavigena* and named as *Cellulomonas* sp. CA-107. In addition, we investigated factors affecting extracellular cellulase production and optimized culture conditions for the cellulase production in a 5-L bioreactor.

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P2-14

Screening of probiotics suitable for fermentation of grains

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Yogurt is preferred by consumers for its unique flavor and health benefits. However, compared with traditional fermented dairy products, probiotics with grains as the substrate has the advantages of high dietary fiber, low fat and low cholesterol, and it is a trend to add it to functional foods. This study first screened probiotics that not only adapt to the human gastrointestinal tract but also was suitable for fermentation of buckwheat. Lactobacilli isolated from healthy human feces and Milk Tofu were tested and screened by tolerance to simulated gastric juice. Probiotic potential was determined based on resistance to simulated gastrointestinal juice and bile salts, antibacterial activity, and cholesterol degradation ability. Results show that 41 bacterial strains with dissolved calcium were obtained from the *Lactobacillus* isolation medium. Five strains with high tolerance to low pH and simulated gastric juice were selected from the screened strains and identified by 16S rRNA gene sequencing. Three strains were *Lactobacillus*, named as *Lactobacillus plantarum* (Lp MT-3), *Lactobacillus plantarum* (Lp MT-

5) and *Lactobacillus salivarius* (Ls AF-7). The tolerance of Lp MT-5 in simulated gastrointestinal juice was the strongest. The bile salt tolerance, antibacterial activity and cholesterol-reducing abilities of Lp MT-3 and Ls AF-7 were both strong.

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P2-15

High-throughput analysis of microalgae cell wall permeability in biotechnological production settings

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Microalgae represent a promising resource for nature-derived compounds in different fields of interest.

The rigid microalgae cell wall (composed of a polysaccharide and glycoprotein matrix) provides the cells with a formidable defence against harsh environments, but typically poses a major bottleneck for efficient product extraction, and hence sustainable process setups.

The aim of this study was to evaluate the applicability of a high-throughput screening method for microalgae cell wall permeability, based on flow cytometry and titration against the exposure time in proceeding fixation steps.

A panel of comparative trials were carried out on *Chlorella vulgaris* cultures to visualize changes in wall permeability via different fixation and staining techniques (methanol treatment and microwaving). Different intracellular targets were addressed (lipid storage vesicles and DNA) to define the optimal strategy with regard to information content on wall configuration and overall robustness.

The tests were carried out for different growth conditions (photoautotrophic and heterotrophic cultures) and process states.

The main results showed that both dies efficiently reached their intracellular target depending on fixation conditions, with minimal non-specific staining. Both assay formats yielded valuable, but non-redundant information on the current wall permeability. As expected, analyses targeted to DNA content proved to be more stable over culture time than those directed to lipid depositories, which can subjected to dynamic changes in response to growth conditions.

The future perspectives will be to implement the flow cytometric assays as a real-time monitoring tool to exert control over product extraction efficiency in different microalgae strains and biotechnological production settings.

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P2-16

***Bizionia beryxiae* sp. nov., isolated from intestinal tract of a splendid alfonsino (*Beryx splendens*)**

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A Gram-stain-negative, non-motile, aerobic and rod-shaped bacterial strain, designated strain RA3-3-1T, was isolated from splendid alfonsino (*Beryx splendens*) collected from the North Pacific Ocean, and subjected to a polyphasic taxonomic study. Strain RA3-3-1T grew optimally at 25 °C, at pH 7.0–8.0 and in the presence of 1.0–3.0% (w/v) NaCl. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences revealed that strain RA3-3-1T belonged to the genus Bizionia, clustering with the type strain of *Bizionia fulviae*. Strain RA3-3-1T exhibited 16S rRNA gene sequence similarities of 98.7, 97.6 and 97.3% to the type strains of *B. fulviae*, *Bizionia paragorgiae* and *Bizionia saleffrena*, respectively, and of 95.5–96.4% to the type strains of the other *Bizionia* species. Strain RA3-3-1T contained MK-6 as the predominant menaquinone and anteiso-C15:0 as the major fatty acid. The major polar lipids detected in strain RA3-3-1T were phosphatidylethanolamine, one unidentified lipid and one unidentified aminolipid. The DNA G+C content of strain RA3-3-1T was 34.1 mol% and its DNA-DNA relatedness values with the type strains of *B. fulviae*, *B. paragorgiae* and *B. saleffrena* were 12–29%. Differential phenotypic properties, together with its phylogenetic and genetic distinctiveness, revealed that strain RA3-3-1T is separated from recognized species of the genus *Bizionia*. On the basis of the data presented, strain RA3-3-1T is considered to represent a novel species of the genus *Bizionia*, for which the name *Bizionia beryxiae* sp. nov. is proposed. The type strain is RA3-3-1T (=KCTC 62140T = NBRC 113024T).

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P2-17

Development of method and apparatus for converting food waste oil into FAME

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Food waste is indeed an untapped resource with great potential for generating energy. Some one-third of all food produced around the world gets discarded uneaten, and environmentalists, energy analysts and entrepreneurs are beginning to take notice. With some 70% of food waste around the world still going into landfills, there is a lot of potential feedstock to keep this environmentally friendly carbon neutral fuel source coming. Food waste consists of 70% liquor content (food wastewater) and 30% solid content. Food wastewater contains an approximately 3–7 g waste oil per 1 L, that the main ingredient of this oil is known as animal or vegetable fats and oils. If you can convert the waste oil containing food wastewater into biodiesel such as fuel, it is expected in the preservation and reuse of waste resource that is greater environmental and economic impacts as much.

FAME (Fatty Acid Methyl Ester) is a biodiesel fuel consisting of long-chain alkyl esters, derived from vegetable oil or animal fat. Biodiesel fuel can be made by chemically reacting lipids with an alcohol, typically methanol or ethanol. Biodiesel fuel can be used in standard diesel engines, either alone or blended with petroleum-derived diesel fuel. Biodiesel fuel can also be used as a low-carbon alternative to heating oil. FAME was produced from a waste oil separated from food wastewater, using catalysts fusion process, to evaluate its potential biomass as a renewable source. In this study, we researched FAME production by the fusion process of enzymatic and acid catalyst. We have produced upper 95% FAME through transesterification reaction using lipase and H_2SO_4 catalyst.

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P2-18

Comparison of the effects of pyrroloquinoline quinone and imidazole pyrroloquinoline

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Pyrroloquinoline quinone (PQQ) is contained in fruits and vegetables such as kiwi fruit and green peppers and in human breast milk. PQQ has diverse effects including an antioxidant effect, a cell growth-promoting effect, and a stimulatory effect on mitochondriogensis. It has a very high reactivity and readily reacts with amino group-containing substances, and it has been reported to change imidazol pyrroloquinoline (IPQ) by forming an imidazole skeleton. It may react with an amino group-containing substance to form IPQ *in vivo*. $PQQNa_2$ is now used as a supplement all over the world. However, the biological activities of IPQ are not known. We investigated whether IPQ functions *in vivo*. A comparative study was conducted to clarify physiological effects including neuroprotective effects, growth-promoting effect and antioxidative effects of PQQ and IPQ using the human neuroblastoma cell line SK-N-SH and the human liver hepatocellular carcinoma cell line HepG2. The expression levels of human COX 4/1 (cytochrome c oxidase subunit IV isoform I) and PGC-1α (PPAR-γ co-activator-1 α), which are indicators of the amount of mitochondria and transcription factor-related mitochondriogenesis, respectively, were measured by RT-PCR. In order to examine the memory-enhancing effects of PQQ and IPQ, measurements were carried out using a step-through-type passive avoidance test. PQQ and IPQ showed cell growth-promoting effect in SK-N-SH cells and HepG2. PQQ and IPQ increased expression levels of COX4/1 and PGC-1α. Both PQQ and IPQ enhanced memory learning ability. These results suggest that IPQ shows almost the same physiological activity as that of PQQ except for antioxidative effect.

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P2-19

Co-culture with endothelial colony-forming cells enhances invasive phenotype of breast cancer cells

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The tumor microenvironment is recognized as a key factor in multiple stages of cancer progression, immune-escaping, and distant metastasis. Endothelial colony-forming cells (ECFCs) in tumor microenvironment are the circulating endothelial precursors that contribute to building new blood vessels in adult body. The present study investigated the effect of surrounding ECFCs on invasive phenotype of MDA-MB-231 breast carcinoma cells by using indirect co-culture technique. Here, we showed that the invasive and migratory abilities of MDA-MB-231 cells were significantly increased by co-culture with ECFCs compared to the cells cultured alone. The invasive and migratory phenotypes of ECFCs were also induced by co-culture with MDA-MB-231 cells. Tube formation of ECFC was enhanced by direct co-culture with MDA-MB-231 cells. Anchorage-independent growth of MDA-MB-231 cells was increased by conditioned media of co-cultured cells. A cytokine antibody array analysis revealed that several cytokines were increased by co-culture which played crucial roles in promoting aggressiveness of breast cancer cells. Taken together, the present study demonstrates that direct and indirect co-culture of breast cancer cells with ECFCs may provide useful information on the effect of tumor microenvironment on breast cancer progression.

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P2-20

Selective extraction of proteins from bacterial cells by electroporation, sonoporation or glass bead homogenization

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Genetic engineering enabled production of recombinant proteins on industrial scale for therapeutic or diagnostic applications in several different heterologous systems, including bacteria. Established processes to extract proteins from bacteria often include use of mechanical forces or chemicals, which cause complete disruption of the cell, resulting in extraction of high levels of contaminants e.g. endotoxins or genomic DNA (gDNA) [1]. In order to improve extraction yields, decrease contaminant loads and decrease downstream process costs new extraction methods have to be developed. One of the promising methods for extracting intracellular products from bacteria is extraction by means of electroporation [2].

Our aim was to compare extraction by means of electroporation, sonoporation and glass beads homogenization techniques for extraction of proteins from *Escherichia coli*. We used monster GFP protein as a model system. Efficiency of extraction methods was compared in terms of target protein recovery, and co-extraction of contaminants (host proteins, endotoxins, gDNA).

Extraction by means of electroporation was superior in terms of ratio between target protein and contaminating host proteins and the decreased co-extraction of host gDNA. We expect that reduction of contaminants achieved by extraction by means of electroporation will lower the number of purification steps in the downstream process and decrease its costs. However, the described method still needs to be optimized in order to increase the yield of target protein molecule.

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P2-21

Variability of microbial communities associated to pecan tree rhizosphere with organic fertilization

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Soil microbial communities are very important in nutrient transformation. The objective of this work was to study variability of microbial communities from the pecan rhizosphere tree with organic fertilization, using as control fertilization with agrochemicals. Soil samples were taken in Chihuahua, México, during April and August 2016 and characterized by heavy metals and their main

components. Five sub samples of each treatment (four in total) were taken and mixed. The microbial analysis was carried out with isolation in general and specific media, DNA total extraction, high-throughput sequencing analysis, total microbial activity by FDA hydrolysis, and community analysis using Biolog MicroPlates. The total microbial activity in April was highest than August in all treatments. In the community analysis, general lineal model showed differences between organic and fertilization with agrochemicals in April, none significant in August. The results of high-throughput sequencing showed 19,719 Operational Taxonomic Units, OTUs were filtered and only 1584 present in the four treatments were used for analysis with Metagenomic Analysis Shaman (Pasteur Institute). The 80.6% of the OTUs was annotated in taxonomic level of gender (185 genders). As additional information, 89 nitrogen-fixing and 42 phosphorus solubilizing strains were isolated and the yield with organic fertilization was lower by 5% with respect agrochemical fertilization.

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P2-22

Screening of lactic acid bacteria feeding for pig, preparing of compound probiotics and its feeding effect on growing pigs

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The study was aimed to screen excellent lactic acid bacteria and develop compound probiotics preparation. The gut tissues of healthy pigs were collected and the lactic acid bacteria were isolated and purified from the gut mucosa by dilution plate method. Through acid and bile salt tolerance experiments, pathogenicbacteria inhibition experiment in vitro and mice safety experiment in vivo, the best strain RS-018 was chosen. After that RS-018 was compounded with P-1 and YB-DY-1 to culture for preparing compound probiotics preparation. According to total viable count, conditions of co-culture and solid-state fermentation (SSF) for compound probiotics were optimized. Preparation of compound probiotics was prepared by aerobic solid-state fermentation with the optimized process parameters. The results showed that a strain of *Lactobacillus reuteri* called RS-018 was screened from ileum, which had strong tolerance for acid and bile salt and could inhibit *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*. Moreover, RS-018 was safe for mice, and even improved their growth performances ($P < 0.05$). The optimum co-culture conditions were as follows: firstly, inoculate 1% RS-018 and 1% P-1 simultaneously, statically culture at 35 °C for 12 h; then inoculate 5% YB-DY-1, 150 r/min, and culture at 37 °C for 24 h. As a result, the total viable count could reach 1.24×109 cfu/ml. The optimum conditions of aerobic SSF were as follows: 5% incubation amount of seed liquid, 60% initial moisture, 36 °C for 36 h,then drying at 35 °C. The results suggested that the total viable count and bacteria type of the compound probiotics preparation meet requirements of compound probiotics for pig. It can be used as feeding compound probiotics for pig.

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P2-23

Screening of copper-enriched microorganisms and optimization of culture conditions

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In the present study, a screening for copper-enriched microorganism from soil with high copper content to convert inorganic copper to microbial copper was performed. A method using sodium diethyldithio carbamate trihydrate as chromogenic agent for determination micro-amounts of copper in cell was developed by spectrophotometry. The efficiency of changing inorganic copper into microbial copper was as a screening index. Screened fungus from the soil was identified by the morphological, based on phylogenetic analysis. The effects of different inoculum size, pH, temperature and culture time on copper accumulation in cell and cell growth were also evaluated. On the basis of single factor experiment, the L9 (34) orthogonal design of the temperature, pH, temperature, the incubation time was used to optimize the process conditions. Our results showed that the strain fungus FT-7 was able to tolerant to copper concentration up to 1000 mg/mL, the best high efficiency for changing inorganic copper into microorganism copper. It belongs to *Aspergillus niger*. Its best transformation conditions were temperature 29.5 °C, pH 5, inoculation amount 4% and culture time 5.5d, the highest conversion rate was 87.65%. *A. niger* is feeding microorganism, can be used as one kind of new organic copper feed additive.

Keywords: Screening, *Aspergillus niger*, Microbial copper, Conversion rate

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P2-24

Prebiotic potential evaluation of starch-based edible films with addition of nystose synthesized by levansucrase from *Bacillus subtilis* natto

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Edible films and coatings can protect foods from deterioration and improve food quality by acting as moisture barrier and as potential vehicles for functional compounds. One of the most recent approaches to improve food quality can be the addition of prebiotics in edible films and coatings. Fructooligosaccharides (FOSs) are oligomers of fructose that are classified as prebiotics by their beneficial effects on the organism, improving the human microbiota specially a nystose (GF3), which consists of a glucose unit (G) connected with fructosyl units (F) at position β-(2 → 1). The aim of this study was to produce by casting starch-based edible films by addition of 10% and 30% of nystose, which was produced by levansucrase of *B. subtilis*. The prebiotic effect through growth of bacteria *Lactobacillus acidophilus*, *L. casei* and *L. plantarum* was then evaluated. The results showed that *L. acidophilus* had a significantly higher growth ($p \leq 0.05$) in 24 h in both films containing 10% and 30% nystose when compared to the glucose media used as control. *L. casei* and *L. plantarum* presented similar results, both had better growth in glucose ($p \leq 0.05$) than nystose, reducing the viability in 12% for *L. casei* and 17% for *L. plantarum*. These results suggest that starch-based edible films added with nystose can be considered promising materials for food packaging, contributing to improve

the nutritional value of the product by exerting its prebiotic effect.

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P2-25

Micropropagation of *Mespilus germanica* L.

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For high frequency in vitro propagation of *Mespilus germanica* L., seed explants were cultured on MS medium supplemented with 6-benzylaminopurine (BA) and α-naphthaleneacetic acid (NAA). A combination of 1.00 mg l^{-1} BA + 1.00 mg l^{-1} NAA was found to be suitable for maximum plantlets height promotion (9.06 cm/plantlet) from seed explants. The maximum leaf number (8.50/plantlet), node number (7.53/plantlet), root number (5.63/plantlet) and root length (7.62 cm/plantlet) was obtained on MS medium supplemented with 2.00 mg l^{-1} BA + 1.00 mg l^{-1} NAA. Related to the effect of BA and NAA on germination percentage and time duration until germination of seeds, maximum germination (86.33%) and minimum time duration until germination (59.00 day) were observed on media enriched with 2.00 mg l^{-1} BA + 0.50 mg l^{-1} NAA and 0.50 mg l^{-1} BA + 1.00 mg l^{-1} NAA, respectively. Plantlets were transplanted to pots filled with perlite and peat (1:1:1:1) for acclimatization. Acclimatized plantlets were planted in bigger pots filled with perlite and transferred to the greenhouse. Upon ex vitro transfer, 100% of plants survived.

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P2-26

In vitro propagation and removal of contaminants in an ornamental orchid (*Phalaenopsis amabilis*)

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A protocol was developed for high frequency in vitro multiplication of an ornamental orchid, *Phalaenopsis amabilis*, using plant growth regulators (PGRs) and silver nano-particles (SNPs). Protocorm-like bodies (PLBs), as explants were cultured on Murashige and Skoog (MS) medium (semi-solid) fortified with various concentrations of kinetin (KIN), indole-3-butyric acid (IBA) and SNPs, either individually or in combination. A combination of 1.00 mg l^{-1} KIN + 1.00 mg l^{-1} IBA + 2000 mg l^{-1} SNPs was found to be suitable for maximum PLBs regeneration (30.40/plantlet), plantlet height (3.83 cm), leaf length (2.76 cm/plantlet), leaf number (5.93/plantlet) and root number (8.36/plantlet) from protocorm explants. The maximum number of plantlet (11.66) was calculated on MS medium supplemented with 1.00 mg l^{-1} KIN + 0.50 mg l^{-1} IBA + 2000 mg l^{-1} SNPs. Also, the highest root length (3.16 cm/plantlet) was obtained on MS medium containing 2.00 mg l^{-1} KIN + 1.00 mg l^{-1} IBA + 4000 mg l^{-1} SNPs. Related to the effect of SNPs on removal of contaminants, maximum contaminant was observed on media without SNPs. Plantlets were transplanted to pots filled with perlite, wood pieces, ionolite and mineral cartridge shell (1:1:1:1) for acclimatization. Acclimatized plantlets were planted in bigger pots filled with perlite and transferred to the greenhouse. Upon ex vitro transfer, 100% of plants survived.

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P2-27

Stem extract of *Basella alba* with potential anticancer and antiangiogenic activity

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Basella alba is an important leafy vegetable of the Basellaceae family, mostly found in the tropical region of Asia and Africa. Various parts of this plant have been used as a medicine from ancient times by Indian and Chinese people for the treatment of a variety of diseases. This plant has been found to be a good source of vitamins, minerals, proteins and several vital antioxidant compounds. In reference to the great ethno-pharmacological properties of this plant species, the present study reports its antioxidant, anticancer and antiangiogenic activity. The methanol extract of *B. alba* stem showed high phenolic and flavonoid content and potent antioxidant activity. It displayed potent cytotoxic activity against Osteosarcoma (MG63) cell line by the MTT assay. Changes in the nuclear morphology of MG63 were observed by DAPI staining and the TUNEL assay confirmed the apoptosis of MG63 cells by DNA fragmentation. An *in vitro* scratch wound healing assay indicated significant inhibition in the migration of Osteosarcoma cells. An *in vivo* antiangiogenesis study indicated the suppression of angiogenesis by inhibition of the growth of blood vessels by the chorioallantoic membrane (CAM) assay. The above results indicate potential nutraceutical properties of the *B. alba* plant that make it a good candidate for the development of effective functional foods.

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P2-28

Production and extraction of a potential antimicrobial biosurfactant by *Bacillus atrophaeus* ATCC9372 cultivated in dairy residues

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Biosurfactants with antimicrobial, antifungal and antiviral properties have been enhancing the potential application in formulations used in several purposes as well as to cosmetics, vaccines, pharmaceuticals, food and even veterinary products. Nevertheless, the high cost of production and purification has been a limitation to the application of biosurfactants. The cost reduction can be obtained using industrial residues once it is able to provide the nutrients necessary and sufficient for high productivity of the biotechnological product. Dairy residues are a byproduct rich in proteins, vitamins, fat, lactose and salts, containing therefore, essential nutrients to cellular growth. Experiments were performed to obtain the ideal lactose concentration of dairy residue culture medium for the best production of the antimicrobial biosurfactant from *Bacillus atrophaeus* ATCC9372. Results indicated that the presence of lower lactose concentration (2.5 g L^{-1}) improve the cell and biosurfactant productivity (Px and PB), respectively, 0.092 $gX\,L^{-1}\,h^{-1}$ and 4.67 $mg_B\,L^{-1}\,h^{-1}$. In addition, the extraction

utilizing ammonium sulfate 5.0 M and t-butanol results in a biosurfactant thin layer that can be re-suspended in buffer and presents an efficient antimicrobial activity from Gram-positive and Gram-negative microorganisms as well as good stability at moderate pH and temperature.

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P3-1

A CRISPR/Cas9 model of sunflower (*Helianthus annuus* L.) resistance for biotic and abiotic stresses

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CRISPR/Cas9 is the most popular gene editing system, currently. It can be used as knocking a gene in to and out of a genome and activating or repressing expression of a gene for all organisms including plants, which are the main source of the food industry. Sunflower (*Helianthus annuus* L.) is a very important oilseed crop in Turkey and in the world. Breeders encounter yield losses, which can reach up to 100%, arised by different biotic and abiotic stresses in different regions of the world each year. Downy mildew, broomrape, stem canker and head rot, and drought, salinity, heat and cold can be given as the examples for biotic and abiotic stresses, respectively. Different methods such as *Agrobacterium*-mediated transformation, electroporation-transformation, particle bombardment and protoplast transformation were used for the delivery of gene editing process to the plant, up-to-date. The decision of the delivery method of plasmid into the host genome is also as important as determination of the proper plasmid. Since many parameters have effects on gene editing processes, in this study, a gene editing model for sunflower resistances is constructed as (i) determination of the suitable genetic changes to perform and design of relevant plasmids, (ii) determination of plasmid transfer method into the sunflower genome, (iii) control for whether the plasmid is transferred and for whether gene editing is effective. This study, which will be the guide for the colleagues both who are working on similar plants, will be the first assembly of CRISPR/Cas9 gene editing and sunflower.

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P3-2

From microinjection to genome editing using zinc finger nucleases and CRISPR/Cas9 system. Characteristics of transgenic pigs generated for xenotransplantation purposes

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The growing shortage of available organs is a major problem in transplantology. The promising solution could be xenotransplantation, i.e., the use of cells, tissues and organs of domestic pig. However, xenogeneic transplantation from pigs to humans involves high immune incompatibility and a complex rejection process. First methods of generation transgenic animals including microinjection were limited and gave only random incorporation of transgene. The rapid development of genetic engineering techniques enables genome modifications in pigs that reduce the cross-species immune barrier using Site-Directed Nuclease technologies (SDN) involving zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and meganucleases. CRISPR-Cas9 (Clustered Regulatory Interspaced Short Palindromic Repeats) is the newest and most powerful of the gene-editing techniques. Here we present transgenic pigs generated using traditional methods and genome editing technologies (ZNF and CRISPR/Cas9). Important issues in producing transgenic animals cover characteristics of transgene, transfection and cell transformation, detection of transgene integration, mapping of transgene, passing to the offspring, homozygote selection, analysis of transgene activity and function. Fully characterized transgenic animals carrying three or more modifications may be used as sources of skin, heart valves or vessels.

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P4-1

Genome sequencing and characterization of novel proteobacterial strains having the ability to use furan aldehydes as only carbon source

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Lignocellulosic biomass is a renewable source of sugars for the biotechnological production of biofuels and fine chemicals. However, the formation of toxic fermentation inhibitors such as furfural and 5-hydroxymethylfurfural (HMF) during acid pretreatment of lignocellulose imposes a significant technical challenge for an efficient bioprocess. The presence of these furans decreases the productivity of microbial strains used for the generation of bioproducts. Consequently, catabolism of furanics is rapidly gaining interest in the biotechnological community having in mind a biodetoxification procedure, relying in microorganisms to degrade these inhibitors. However limited information about furans biodegradative pathways and their encoding genes is available, mostly due to the fact that few organisms have been identified which completely metabolize furfural or HMF. In this work we reported the genome sequencing of twenty novel proteobacterial strains having the ability to use furfural and/or HMF as the only carbon and energy source. All the strains were isolated from activated sludge treatment of pulp mill wastewater and most of them were classified in the *Pseudomonas* genus according their 16S rRNA-encoding genes. Most remarkable is that several of these strains showed a more efficient catabolic phenotype for turnover of furans than previously reported bacteria, including faster growth and increased tolerance to high concentration of substrate. Genome sequencing of these strains has revealed the close phylogenetic relationships of the furans-degradative encoding genes and a broad diversity of gene cluster organizations. The further characterization of the furans biodegradative pathways in these strains holds great

promises for their application in biodetoxification of lignocellulosic hydrolysates.

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P4-2

Withdrawn

P4-3

Molecular characterization of *Rhodeus uyekii* tripartite motif protein 1 (TRIM1) involved in IFN-γ/LPS-induced NF-κB signaling

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The tripartite motif-containing (TRIM) proteins are involved in a wide range of cellular processes, and the role of TRIM1 in immunity has been explored. However, fundamental studies on fish TRIM1 are lacking. In this study, we cloned and characterized TRIM1 cDNA from the Korean rose bitterling, *Rhodeus uyekii* (RuTRIM1). Two RuTRIM1 isoforms (RuTRIM1-X1 and RuTRIM1-X2) were identified. The coding sequence (CDS) of RuTRIM1-X1 comprised 2157 bp encoding a 718-aa protein, and the CDS of RuTRIM1-X2 comprised 1929 bp encoding a 642-aa protein. Both RuTRIM1 isoforms contained a RING finger domain, B-box 1, B-box 2, B-box C-terminal domain, COS box, FN3 motif, and PRY/SPRY domain. The deduced RuTRIM1-X1 and RuTRIM1-X2 proteins had high amino acid identity (76.27–98.89%) with orthologs from various other species, and a phylogenetic tree was constructed. RuTRIM1-X1 and RuTRIM1-X2 mRNA were expressed in all *R. uyekii* tissues tested, with the highest expression levels detected in the hepatopancreas. RuTRIM1-X1 mRNA levels were stable across all early developmental stages, whereas RuTRIM1-X2 mRNA expression was detected at 1 day post-fertilization (dpf) and moderately increased until 21 dpf. An in vivo ubiquitination assay showed that RuTRIM1 exhibited RING-dependent E3 ubiquitin ligase activity, mainly by comparing RuTRIM1-X2 to RuTRIM1-X1. The subcellular localization of the two RuTRIM1 protein isoforms was characterized, revealing that they formed aggregates in cytoplasmic bodies in Raw264.7 cells. Interferon-γ/lipopolysaccharide-induced nuclear factor-κB signaling was negatively regulated by RuTRIM1-X1 and RuTRIM1-X2, and the negative effect was reversed in RING deletion mutants. To our knowledge, this is the first study to characterize fish TRIM1, which may play a role in the inflammatory response.

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P4-4

Comparative analysis of nuclear and mitochondrial genomes of flor yeast strains

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Flor yeast strains represent a specialized group of *Saccharomyces cerevisiae* yeasts used for biological wine ageing. Using a combination of PacBio and Illumina NGS platforms we have generated high-quality nuclear and mitochondrial genomic sequences of 3 flor strains from Russian culture collection. SNP analysis versus available genomes of wine and flor strains identified 2270 flor-yeast-specific genetic variants in 1337 loci. Gene ontology analysis in combination with gene content evaluation revealed a complex landscape of possibly adaptive genetic changes, related to genes associated with cell morphology, mitotic cell cycle, ion homeostasis, DNA repair, carbohydrate metabolism, lipid metabolism, cell wall biogenesis etc. Pangenomic analysis discovered the presence of several well-known "non-reference" loci of potential industrial importance, deletions of clustered genes involved in maltose and

asparagine utilization, iron uptake with some clear metabolic and phenotypic effects.

Mitochondrial genomes of analyzed strains show high degree of conservation of coding and noncoding sequences, identical gene order and unique exon-intron organization of *cox1* genes. Reduction in the relative abundance of mobile GC-clusters, direct and inverted repeats, active *ori* sequences in flor yeast mtDNAs as compared to wine mitogenomes suggests that these genome destabilizing structural elements are counter-selected to ensure mtDNA integrity in the oxidative and mutagenic environment of biological wine aging. Statistical analysis of the character of SNP variation in flor yeast mitoprotein CDS suggests that these loci are also under stabilizing selection.

Our study provides new insights in the nature of genetic variation in flor yeast strains, contributes to in-depth understanding of their evolutionary history.

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P4-5

Starch phosphorylase genes in *Solanum* species: identification, variability, and expression

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In the plant, starch (α-glucan) phosphorylase (Pho) is the key enzyme catalyzing the starch phosphorolysis. In *Solanum*, the plastid Pho1 and cytosolic Pho2 affect the fruits/tubers development. The objective of this study is to conduct a comparative genomics analysis of starch plastid phosphorylase genes in *Solanum* species. *Pho1a* genes from wild and cultivated tomato and potato species were cloned and sequenced for the first time. The identified tomato *Pho1a* genes (8029–8351 bp) were almost half the length of *S. tuberosum Pho1a* (16 kb), due to the TST mobile element lack in the intron V. Potato and tomato genes and encoded proteins showed high homology, including tertiary structures with characteristic N- and C-terminal domains. The tomato *Pho1a* genes demonstrated a low polymorphism level (4.63%), and a roughly 3-fold difference in variability between green- and red-fruited (GF and RF) species (3.58% vs. 1.24%). Twenty of the 55 non-synonymous SNPs were predicted to be deleterious. Amino acid variability divided tomatoes into two groups differed in fruit color and sugar composition. The tissue-specific *Pho1a* genes expression analysis was performed in species representing both groups. In the RF tomatoes, the fruit-specific *Pho1a* expression profile was correlated with starch degradation dynamics during fruit ripening. In the GF species, the difference between mature green and ripe fruit *Pho1a* expression was insignificant, which may indicate changes in fruit starch metabolism. The results can help clarify the mechanism of starch degradation, and can be used in *Solanum* biotechnology. The study was granted by the RFBR (17-29-08017).

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P4-6

The mechanism of natural competence loss in animal pathogen *Streptococcus zooepidemicus*

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The natural competence is the genetically specified ability of some bacteria to alter their genome by taking up foreign extracellular DNA from its environment. Initially it was found in *Streptococcus pneumonia*, later it was described for many species of Streptococci genus and for other groups of bacteria. The mechanism of natural competence is well studied and is similar for many bacteria. Interestingly, several strains of well-known animal pathogen *Streptococcus zooepidemicus* lack this feature.

The present study addresses the genetic background of natural competence loss in selected strains of *S. zooepidemius*. The genomes of studied strains contain all genes needed for either early or late phases of natural competence occurrence. However, the point mutation in the gene encoding comEA DNA-binding protein leads to the frame shift and loss of protein activity. The comEA protein is a transmembrane protein that binds to the foreign DNA and provides its transport through the membrane into the cytoplasm. The experiments on natural competence induction by comX overexpression followed by successful transformation with the linear PCR fragments confirmed that the frame-shift mutation in comEA gene is the only reason of the loss of natural competence in *S. zooepidemicus*.

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P4-7

Complete genome sequence of *Bifidobacterium choerinum* FMB-1, a granule starch degrading bacterium

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The strain *Bifidobacterium choerinum* FMB-1 was isolated from rumen fluids of Korean native cattle (*Bos taurus coreanae*) and it has granule starches degrading ability. Approximately 80% of native granule starch was degraded within 8 h. Here, we performed genome sequencing and reported the first complete genome of this strain, which consists of a single, circular chromosome (2,257,294 bp) and plasmid (11,012 bp) with 65.5% average G+C content. Genome analysis revealed that at least 11 protein-coding genes were related to α-glucan degradation. The abundance of these genes may affect the efficacy of granular starch degradation. Also, it was additionally identified to have antimicrobial resistance gene not found in other *B. choerinum* genomes. The genome information of *B. choerinum* FMB-1 could provide a better foundation for further studies of RS degradation related strains and its degradation mechanism.

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P5-1

The use of recombinant lectins for the bioanalysis of cell surface glycosylation

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Biological glycosylation is the process which adds specific sugars to other sugars, proteins and lipids. Protein glycosylation is one of the most important post-translational modifications, which occurs in more than half of all proteins present in the human body. Abnormal glycosylation has been demonstrated to be linked to many different diseases due to alterations associated with protein folding and biological function. Therefore, glycosylation is absolutely essential for the correct structure, function and stability of important proteins.

Surface glycosylation patterns play a key role in the modulation of the immune responses which are mediated by carbohydrate-binding proteins called lectins. Such biomolecules are typically highly selective for specific glycan structures, making them extremely useful for glycan variation investigation.

A rapid and accurate bioanalytical method to detect early unhealthy cell signs during a bioprocess is a current issue facing the industry. It is widely known that as cells become stressed or diseased the earliest changes that occur are in cell surface glycosylation.

CHO cells are the host cells of choice of the rapidly emerging biopharmaceutical industry for the production of glycoprotein therapeutics. Hence, this research work intends to investigate the interaction between recombinant lectin probes with the membrane glycoconjugates of normal CHO cells and compare this to CHO cells that have been subjected to several stressing conditions.

The aim is to develop a rapid and accurate bioanalytical method based mainly on flow cytometric analysis to monitor the bioprocessing cell health by interrogating its surface glycosylation with unique probes.

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P6-1

Benefits of vitamin B5 regulation on CHO cells energy homeostasis and therapeutic production

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Background and novelty: Vitamins are essential micronutrients required to support the growth and propagation of any living cell. Indeed, mammalian cells cannot synthesize them, and the lack of vitamins in the diet is directly linked to severe cellular defects [1–3]. Changes in central metabolism limits growth and recombinant protein expression highlighting a regulatory link between cell metabolism, metabolite consumption and accumulation and cell growth [4].

Experimental approach: We designed an improved selection method based on the co-expression of vitamin B5 intracellular transporter, relying on mammalian cell dependence on this vitamin for energy production. We deciphered the molecular and metabolic changes in the resulting B5-selected populations, and chemically engineered recombinant cells with improved energy homeostasis.

Results: The B5-selection method yields polyclonal cell populations producing recombinant proteins at homogeneous and high level, using the selective advantage of improved cell metabolism, growth and viability. This method is also efficient to recover variant cells synthesizing difficult-to-express chimerical proteins at elevated levels, unlike state-of-the-art procedures. Understanding the associated metabolic changes led us to produce cells with increased viability and hence recombinant protein productions.

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P6-2

Introduction of single-use multi-bioreactor system with full control functions for pH, dissolved oxygen, and temperature of individual bioreactors

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A variety of biopharmaceuticals such as antibodies, proteins, and gene/cell therapeutics have been developed in recent decades and demands for them have increased dramatically every year. To meet such demands, it is essential to cultivate cells that produce biopharmaceuticals in large-scale bioreactors and it is very important to optimize culture conditions for massive production of biopharmaceuticals. Scaling-down technology, using multi- and small size (less than 100 mL) bioreactors, has been an effective tool for applying DoE (Design of Experiment) and QbD (Quality by Design) for process optimization of large scale cell cultures.

In this study, a newly developed single-use multi-bioreactor system that can control the pH, DO, and culture temperature of each bioreactor (currently 6 bioreactors, which is expandable to 24 bioreactors in a multi-bioreactor culture system) is introduced. The bioreactors in the single-use multi-bioreactor system are small (working volume is up to 30 mL) but enough for cell culture process optimization that requires multiple samplings. Each bioreactor is capable of controlling the values of pH, DO and temperature. In particular, to minimize the use of peristaltic pumps that is essential to add the base solution for control of pH, a new pneumatic pulsing system for addition of base solution is developed and applied, resulting in enhanced simplicity of tubing connections in bioreactors. Additionally, since the culture vessel is single-use, there are well-known advantages of single-use bioreactor systems such as unnecessity of in situ sterilization procedure, lower risk of contamination and lower cost compared with conventional stainless steel culture vessels.

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P7-1

Stem cell fate: extracellular glutamine shock for identification of key metabolic influencers in pluripotent and neural stem cells

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Metabolic pools are regulators of cell phenotype by affecting several cellular components such as protein, DNA and histones, and through several mechanisms such as allosteric regulation of enzymes or methylation of DNA. This diversity of targets and mechanisms partially explains the current impossibility to mathematically model the metabolic influence on cell phenotype. However, identification of the key metabolic influencers provides hope for devising targeted approaches to manipulate stem cell fate for improving expansion or differentiation efficiency and economics. In this work, we propose a new way to systematically identify metabolic pools that regulate stem cell fate.

Our hypothesis is that the most important metabolic pools are the ones whose intracellular concentrations are more tightly controlled. Thus, we applied principles of process dynamics and control to human pluripotent stem cell lines (hiPSC) and human neural stem cell lines (NSC) challenged with a step increase in extracellular glutamine. Intracellular metabolomes of hiPSC and NSC were extracted and analysed by LC–MS/MS before and after the glutamine challenge. By modelling the metabolic dynamic profiles and determining control characteristics of settling time and damping coefficient, we have identified the most controlled metabolites in each cell type. Finally, by selecting the most controlled metabolites as targets, we will perturb their intracellular pools to confirm their importance in stem cell homeostasis through step experiments. If successful, this work establishes a novel, systems-level approach for the identification of key metabolites which could be critical for the design of targeted approaches for stem cell processing.

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P7-2

Withdraw

P7-3

Anaerobic succinate fermentation in D-glucose PTS-deficient *E. coli* cells

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Under anaerobic conditions, bacterial cells convert sugars to various fermentation products including succinate, lactate, acetate, and ethanol to regenerate oxidized coenzymes such as NAD^+, and also to obtain biochemical energy (i.e. ATP). The fermentation pat-tern or metabolic pathway of certain bacterium is optimized for anaerobic cellular growth, as programmed in the genome that is a result of long-term microbial evolution. It was observed that *ptsG* mutant cells of *E. coli* K-12 strains suffered from poor growth in D-glucose containing fermentation medium, soon afterwards adapted to grow rapidly in the same batch culture. Metabolite anal-ysis and genome sequencing showed that mixed acid fermentation was changed to succinate fermentation via specific mutations in transcription factors capable of turning on and/or off gene expres-sion of sugar transporters. These results indicate that fermentation patterns can be regulated elaborately by the expression of sugar transporters in the cells.

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P7-4

Study of acetate metabolism using different carbon and nitrogen sources in *Escherichia coli*

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Escherichia coli, *E. coli*, is a model organism in biological and biotechnological processes. Thus, *E. coli* is used in many industries for the production of high-interest compounds [1]. Due to the large number of processes that are based on the use of this bacteria, the study of its central metabolism and regulation is essential to optimize all these biotechnological processes.

Furthermore *E. coli* is capable to grow using diverse carbon and nitrogen sources, and them are relevant factors for the *E. coli* metabolism fluxes [2,3].

This work is focused on the study of acetate metabolism of *E. coli*. *E. coli* BW25113 and deficient strains involved in acetate metabolism are grown in minimal medium MM9 and in complex medium TB7 (which are nitrogen sources based in inorganic ammonium and peptides, respectively), supplemented with glucose and glycerol as a carbon source.

Under these conditions growth rate, protein lysine acetylation and intracellular concentration of acetyl and succinyl donors such as acetyl-phospate, acetyl-Coenzyme A and succinyl-Coenzyme A, have been evaluated. The data of growth rate revealed a dependency with the carbon and nitrogen source employed. In addition, concentration of acetyl and succinyl donors, and protein acetylation are also influenced by carbon and nitrogen sources.

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P7-5

Physiological characterization of metabolically engineered *E. coli* K4 strains with improved pathways for capsular polysaccharide biosynthesis

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Among capsulated bacteria, some produce polysaccharides with unique properties that have been shown to possess relevant industrial applications and commercial value. *Escherichia coli* K4 natively produces a capsular polysaccharide that shares similarity with chondroitin sulphate (CS), in fact, both molecules are composed of alternating glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc) β-linked residues, and differences among the two polymers regard the presence, in the microbial molecule, of an acid-labile fructose on the C3 of GlcA, and the absence of sulphate groups in various positions. Recent efforts focused on the development of genetic and fermentation strategies to increase its production titers up to technologically attractive levels. However, the control of the metabolic pathways leading to CPS synthesis together with the effect of varying the concentration of pathway intermediates on CPS final titers, is still quite unexplored and not fully understood. In the present study genes involved in the biosynthesis of UDP-sugar CPS precursors, were overexpressed in the wild type *E. coli* K4, and the recombinant strains were characterized at the physiological level to study their effect on the biosynthetic machinery. Shake-flask and small scale fermentation experiments were performed to gain insight into the strains metabolism. In particular, the gene coding for UDP-glucose dehydrogenase that is involved in the biosynthesis of UDP-GlcA seemed to play a central role in affecting CPS production.

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P7-6

Gene disruption to improve 1,3-propanediol production in *Citrobacter braakii* strain TB-96

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The use of biodiesel fuel (BDF) as an energy source to replace fossil-fuels is gaining attention worldwide. However glycerol waste, by-product of BDF production, is highly alkaline and contains impurities which has made refining and utilization difficult. Therefore, at present, it is incinerated at cost. 1,3-Propanediol (1,3-PD) is a platform chemical that can be produced from waste glycerol by microorganism fermentation. *Citrobacter braakii* TB-96 previously isolated in our laboratory is a promising strain with fast growth rate and high 1,3-PD productivity. The disadvantage of this strain was that growth stops at log phase, and productivity of 1,3-PD decreases consequently. In present study, we genetically modified strain TB-96 to improve production of 1,3-PD through disruption of lactate dehydrogenase (*ldh*) and pyruvate-formate lyase (*pfl*). The former is main by-product in glycerol fermentation while the latter is a toxic by-product that disrupts growth of microorganism. We also performed 1,3-PD production experiment using TB-96 Δ*ldh*Δ*pfl* in 1.5 L scale of fed-batch bioreactor. Using pure glycerol, tshe wild type TB-96 strain accumulated 56 g/L of 1,3-PD in 60 h while mutant strain achieved 74 g/L, which was 1.32-fold higher than the parent strain.

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P8-1

Novel β-glucosidase retrieved from the south-eastern Brazilian Secondary Atlantic Forest soil metagenomic library

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Beta-glucosidases are key enzymes involved in sugarcane degradation for bioethanol production from lignocellulosic biomass which complete the final step during cellulose hydrolysis by converting the cellobiose to glucose. In this sense, metagenomics has become a powerful tool for accessing and exploring the biological and molecular biodiversity present in different natural environments. Here, we employed a functional metagenomic approach to exploit the enzymatic potential of a soil enriched with decaying plant matter from a Secondary Atlantic Forest region, using the optimized and synthetic pSEVA232 broad host-range vector for library production. Screening of the metagenomic library resulted in the identification of a novel β-glucosidase, presenting low sequence identity to other known glycoside hydrolases (59% of amino acid identity). The purified enzyme was most active in pH 5.5 at 50 °C and showed hydrolytic activity toward various *p*NP substrates containing β-glucose, β-galactose, β-xylose, β-fucose and α-arabinopyranose as well as toward cellobiose. Furthermore, β-glucosidase activity was improved by 10 mM of divalent cations Mg^{2+}, Co^{2+}, Ca^{2+} or NaCl. In addition, Lfa2 showed high glucose tolerance, exhibiting 20% of remaining activity in 800 mM of glucose. Synergistic effect of β-glucosidase on GH5-CBM3 cellulase from *Bacillus subtilis* 168 activity was measured using carboxymethyl

cellulose (CMC) as substrate, presenting an increase of about 1.5-fold by the addition of β-glucosidase when compared with the endoglucanse alone (63% increase in total glucose released). These results indicated that β-glucosidase Lfa2 have a great potential to complement commercial enzyme cocktails to produce fermentable sugars from lignocellulosic biomass.

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P8-2

Systematic analysis of the microbial community in a fixed-bed biofilm reactor for oil sands process water (OSPW) treatment

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Metagenomic and metatranscriptomic sequencing analyses were performed to investigate the microbial community in a fixed-bed biofilm reactor for raw oil sands process water (OSPW) reclamation. 16S rRNA and 18S rRNA gene segments targeted metagenomic sequencing showed that *Proteobacteria* and *Environmental* were the dominant bacterial and fungal phylum during the establishment of the biofilter. The dominant bacterial class on the top, middle and bottom of the bioreactor was determined as *Alphaproteobacteria* (36.4%), *Alphaproteobacteria* (30.0%) and *Saprospirae* (24.4%), indicating that the reclamation of OSPW was achieved by the aerobic and anoxic combined biodegradation processes. The established fixed-bed biofilm reactors were used for the treatment of raw and ozonated OSPW (utilized ozone dose of 30 mg/L) by re-circulating OSPW through the biofilter for 8 times with a total hydraulic retention time (HRT) of 16 h. Metagenomic sequencing analysis showed that *Proteobacteria* and *Environmental* were still the dominant bacterial and fungal phylum in the biofilter after treating ozonated OSPW. *Alphaproteobacteria* was the most abundant bacterial class in the biofilter after the treatment of raw and ozonated OSPW, the percentage of which was 23.9% and 16.9%, respectively. Metatranscriptomic sequencing analysis found that the functional abundance of the aromatic compounds metabolism and organic acids degradation pathway was improved from 0.05% and 0.29% in indigenous microbial community to 0.76% and 0.38% in the microbial community of the biofilter. The microorganisms from the *Proteobacteria* and *Environmental* phylum may play an important role in the OSPW reclamation through the organic acids degradation and aromatic compounds metabolism pathway.

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P9-1

Synthesis of fatty acid ethyl esters in engineered *Yarrowia lipolytica*

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Recent advances in the production of biofuels by microbes have attracted attention due to increasingly-limited fossil fuels. Biodiesels, especially fatty acid ethyl esters (FAEEs), are considered a potentially fully-sustainable fuel in the near future due to similarities with petrodiesels and compatibility with existing infrastructure. However, biosynthesis of FAEEs is usually limited by the supply of precursor lipids and acetyl-CoA. In the present study, we explored the production potential of an engineered biosynthetic pathway coupled to the addition of ethanol in the oleaginous yeast *Yarrowia lipolytica*. To construct the FAEE synthesis pathway, wax ester synthase (WS) encoding genes from different species were codon-optimized and heterologously expressed in *Y. lipolytica* and the most productive engineered strain was found to express a WS gene from *Marinobacter hydrocarbonoclasticus*. FAEE synthesis was then improved by optimization of the promoter of WS overexpression, elimination of β-oxidation, and redirection of carbon flux towards acetyl-CoA synthesis. The new engineered strain, coupled with an optimized ethanol supplementation, could synthesize a maximum extracellular FAEE titer of 1.2 g/L in shake flask cultures, approximately 6-fold of that produced by the wild-type strain. This study demonstrated that an engineered *Y. lipolytica* strain possessed a high capacity for FAEE production and may serve as a platform for more efficient biodiesel production in the future.

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P9-2

Bacillus subtilis overproduces industrially important extracellular enzymes upon the targeted deletion of bacilysin biosynthetic operon

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Bacilysin being produced by *Bacillus subtilis* is the smallest peptide antibiotic ever known. It is composed of an N-terminal L-alanine and a modified amino acid at its C-terminal, namely anticapsin. *bacABCDEF* operon and a monocistronic gene bacG are functional for bacilysin production in the organism, *bacABCDFG* being needed for the flux from prephenate to anticapsin and then to mature bacilysin while bacE gene within the operon is involved in resistance of the producer by pumping bacilysin out of the cell. Our earlier studies demonstrated that quorum sensing global regulation operates in bacilysin biosynthesis through the action of ComQ/ComX, PhrC (CSF), ComP/ComA and molecular regulation also requires an intact surfactin biosynthetic operon, srfA. We recently performed a dynamic secretome analysis of *B. subtilis* PY79 and its bac operon-deleted derivative OGU1 by taking 2DE MALDI TOF/MS and LC–MS MS as complementary approaches and identified ca. 200 proteins (extracellular, membrane and wall-associated proteins) differentially expressed between two strains. Since *B. subtilis* is one of the most important cell-factories with a significant capacity to produce a wide range of extracellular enzymes, of biotechnological interest was a significant increment in levels of the industrially-important extracellular enzymes upon the deletion of bac operon. These enzymes included chitosanase, arabinanase, levanase, lipase, phytase, endonuclease, bacillopeptidase F and minor extracellular protease. In this report, the results of quantitative transcript analysis of the respective csn, abn2, sac, estA, phy, yhcR, bpr and vpr genes as well as enzymatic activities of their products are presented.

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P9-3

$NADP^+$-dependent malic enzymes as a tool for improving oil production in *Rhodococcus* bacteria

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Rhodococcus bacteria are able to produce triacylglycerols (TAG) from diverse carbon sources, including industrial wastes. Oleaginous bacteria may serve as a source of lipids with potential application in the industry, such as in the production of biofuels,

biolubricants, additives for cosmetics or feed, oleochemicals and other manufactured products. Thus, rhodococci could serve as lipid production platforms that could be rationally optimized via genetic engineering. Based on the well-known malic enzyme (ME) participation on lipid metabolism in several eukaryotic microorganisms, herein we investigated the occurrence and natural contribution of these enzymes in different Rhodococcus strains. Enzymatic activity measurements and the use of a metabolic inhibitor (sesamol) indicated that NADP+-dependent ME's are contributing to the TAG biosynthesis as well as cell biomass production in the oleaginous *R. jostii* RHA1 and *R. opacus* PD630 strains. In addition, we functionally characterized a putative ME encoded by the RHA1_RS44255 gene in *R. jostii* RHA1. Heterologous expression of RHA1_RS44255 in *E. coli* BL21 (DE3) resulted in a two-fold increase in NADP+-ME activity. Over-expression of the gene in RHA1 and PD630 strains grown with glucose, promoted an increase up to 10% by cellular dry weight in total fatty acid production without sacrificing cellular biomass. Expression of this gene in *R. fascians* F7 resulted in an increase of 1.3–1.4-fold in lipids during cultivation on glycerol. Results confirmed the contribution of NADP+-ME on TAG accumulation in oleaginous rhodococci and the utility of these enzymes as an alternative approach to increase bacterial oil production from different carbon sources.

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P9-4

Improving the stress tolerance of the oleaginous yeast *Lipomyces starkeyi* for industrial purposes

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An emerging potential alternative for biodiesel production is represented by microbial lipids, also referred as single-cell oils (SCOs), which could lead to a green and sustainable biodiesel production process, with no competition with the food supply chain. Many microorganisms belonging to the genera of algae, bacteria, yeast and fungi can accumulate lipids under specific cultivation conditions. Among them, the utilization of oleaginous yeast is advantageous due to fast growth rate and high oil content compared to algae.

A sustainable production of SCOs implies the utilization of lignocellulosic biomasses as substrate for growth. Unfortunately, during their hydrolysis different inhibitory molecules are formed, limiting cell growth and, consequently, SCOs production. The development of robust cell factories is therefore crucial for the establishment of sustainable processes. Strategies to improve the stress tolerance of the oleaginous yeast *Lipomyces starkeyi* will be described, together with their effect on SCOs production.

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P9-5

Display of white spot syndrome virus protein VP28 on the surface of *Lactobacillus plantarum* for oral vaccination in shrimp

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White spot syndrome virus (WSSV) is one of the most serious pathogen for shrimp and crustaceans in worldwide, which causes the formation of white spot in the exomesoderm and makes severe damage to shrimp culture industry. The 28 kDa envelope protein VP28 which plays a key role in initial infection is considered as a potential candidate for vaccine against the virus. In this study, we aimed to create probiotic strains which can be used as a WSSV oral vaccine by displaying this protein on the cell-surface of *Lactobacillus plantarum* WCFS1. Here, the VP28 protein was fused to various cell wall anchors and the resulting fusion protein was expressed in *L. plantarum* WCFS1 under the control of pSIP expression system. To develop non-GMO vaccines, this protein was fused to lysM motifs, expressed in *E. coli* before anchored to *L. plantarum* WCFS1. The results showed that the recombinant VP28 can be attached to the cell wall either via C-terminal cell wall anchors or N-terminal lysM motifs. The localisation of them on the bacterial cell surface was indicated by flow cytometry or immunofluorescence microscopy, while the expression was confirmed by western blot. The success in display of VP28 protein on the surface of a food grade organism can become an attractive strategy to produce VP28-based oral vaccines to against the WSSV infection. The novel vaccines have been applied in shrimp cultivation via oral administration.

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P10-1

High ACC deaminase producing copper and nickel tolerant rhizobacteria enhances metal tolerance and seedling growth of Indian mustard plant

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Copper influenced serpentine soils are usually nutrient deficient and presence of high concentration of metals creates detrimental effect on plant growth. Plant growth promoting (PGP) rhizobacteria are one of the major factors of plant and soil productivity in stressed conditions. Rhizobacteria from *Trifolium pratense* L. growing in vicinity of a century old active Cu smelter (Karabash, Russia) were isolated and analyzed for their different heavymetal tolerance, antibiotics resistance and PGP attributes. The soil was found serpentine in nature with high concentration (mg/kg) of Cu:2283, Ni:485, Cr:744, Pb:904 and Zn:851. Out of 26 Cu and Ni tolerant (100 mg/L each) bacterial isolates, two strains namely *Pseudomonas* sp. TR15a and *Bacillus aerophilus* TR15c showed maximum tolerance against Cu:1750; Pb:3000, Cr:1000, Cd:750, while Ni (1750 mg/kg) was highly tolerated by strain TR15a and consortium in agar plate. Both strains and their consortium also showed resistance against varied antibiotics (ampicillin: 10 μg, kanamycin: 30 μg, chloramphenicol: 30 μg, penicillin: 10 IU, tetracycline: 30 μg and streptomycin: 30 μg). Strain TR15a showed higher PGP activities compared to strain TR15c, whereas, their consortium showed maximum activities with ACC deaminase

(53745 nM α-keto mg/protein/h), indole-3-acetic acid (7.92 mg/L), siderophore (12 mm) production and P-solubilization (315 mg/L). For seedling test, sterile seeds of *Brassica juncea* L. inoculated with 10^{-7} CFU inoculum were germinated in 75 mg Cu/Ni per Kg enriched sterile soil for 14 days. Study suggests that consortium outperformed with higher percentage of seed germination (98%), root length (62%) and shoot length (38%) compared to control which can help in reclamation of Cu and Ni rich derelict sites.

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P10-2

Entamoeba cyst wall synthesis: a new insight

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Entamoeba histolytica, causative agent of amoebiasis infects human through chitin walled cyst. This makes encystation an excellent target for development of new drugs aimed at preventing spread of *E. histolytica*. Since *E. histolytica* cannot be encysted in vitro, it reptilian counterpart, *E. invadens* is used to study encystation as their cysts have similar characteristics. Two of the most important characteristic of cysts are the chitin wall and the presence of four nuclei. Here we studied the development of these two characteristics develop as the encystation proceeds. In vitro encystation is asynchronous and completes within a time period of 72 h. The cyst wall of *Entamoeba* is consisted of carbohydrate moieties like Chitin, Chitosan and carbohydrate binding lectins like Jacob, Jessie and Chitinase. During the early phase of encystation, Chitin is synthesized and gets deposited on the surface of encysting *Entamoeba*. The penultimate enzyme of the Chitin synthesis, Chitin synthase has been cloned and characterized. The Jacob lectin gets cross-linked with the Chitin fibrils and finally the deposition of Jessie makes the wall impermeable to even small molecules. By 12th hour immature cysts at different stages of wall formation was observed. The wall formation appears to be starting from a single spot and then spreads all over the surface of the cell. The chitin wall formation is mostly completed by 24th hour. But at this stage the cysts still contains only a single nucleus. The nucleus starts dividing after 24th hour. 48th hour encystation culture cysts are mostly with one or four nuclei and a few are with two or three nucleus.

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P10-3

The effects of high-voltage electric field on microbial communities in paddy soil

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Power industry has intensively developed to support rapid urbanization and growing economy. Electric power transmission lines and sub-stations are continually expanding with elevated level of voltage, which can lead to increased electric field strength in the surrounding space and consequently may have ecological impact on associated soil biota. However, the effects of high voltage electric field on soil microorganisms remain unexplored. In the present study, we conducted a mesocosm experiment to investigate the impact of simulated electric field (50 Hz, 8 kV/m) on the diversity and function of paddy soil microbiota. The 63 day exposure did not change soil respiration, measured soil properties and extracellular enzyme activities, except that urease activity decreased significantly after 7 days of exposures. The main results of this study were as follows: (1) it was revealed that soil urease activity decreased significantly after 7 days of exposures, however it diminished gradually versus control. (2) Results of real time PCR showed that denitrifying gene abundance (at DNA level) had been affected by the electric field. (3) Illumina high through-put sequencing showed that microbial community structure was slightly affected at DNA level. The microbial α-diversity did not changed compared with control group. Taken together, these results demonstrated that the presence of an electric field of 50 Hz 8 kV/m will not influence the composition and physiology of soil microbial communities, thus endorsing its mutual effect on 'soil microbial health'.

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P10-4

Improving thermal resistance of probiotics through modifications in the fermentative process using surface response methodology

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Probiotic strains are used as nutraceutical ingredients. However, these bacteria could lose their viability after thermal downstream treatments, such as stabilization using spray drying. Different strategies for increasing thermal resistance of probiotics have been used with different outcomes, involving changes in the conditions of the downstream process or the addition of thermal protectors, like defatted milk, which may trigger allergy or intolerance problems on the final consumer. Other strategies applied to probiotics are related to the fermentative process and they may increase the thermal resistance the bacteria thanks to the modifications in the cell wall produced when the strains are forced to face different kinds of stress.

In this work, a strategy to increase the thermal resistance of a probiotic strain (*Lactobacillus casei* CECT 475T) was developed, by forcing the strain to face different forms of osmotic and thermal stress. In this new approach, both factors were applied independently and combined, with the final aim to increase the viability of the cultures after spray drying.

Surface Response Methodology (SRM) was used for the design of the experiment. The study was done using a 3-level factorial experimental design and studied the effect of 2 variables (osmotic and thermal stress) in 9-runs with triplicates. As response variables, the viable cell counts after the fermentative process and the spray drying were measured. The aim was to identify the optimal fermentative conditions for improving the thermal resistance of the strain, changing only the variables related to the fermentation process and avoiding the use of allergenic protective carriers.

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P10-5

Molecular characterization of iron–sulfur cluster regulator binding motifs in pathogenic bacterium *Pseudomonas aeruginosa*

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Iron–sulfur cluster, Fe–S is a key cofactor of several proteins required for various cellular functions, including respiration, metabolism, nitrogen fixation, RNA modification, and gene regulation. In *Pseudomonas aeruginosa*, IscR is globally dimeric transcriptional regulator in Fe–S biogenesis, modulates a cellular iron homeostasis, and responds to environmental stresses including oxidative stress from the host immune system. The *P. aeruginosa* PAO1 contains a stress-induced isc gene cluster consisting of iscRSUA-hscBA-fdx2-iscX. In this study, site-directed mutagenesis of was conducted and the results showed that cysteine and histidine residues involved in Fe–S ligation and regulatory mechanism of IscR. The Fe–S containing protein in mutated IscR was decreased compared to that in wild-type IscR. The results from in vitro binding assay illustrated that the ligation of Fe–S was required for repression mechanism of IscR and holo-IscR bound to two IscR binding sites located on the isc operon's promoter. The sequence-specific binding of IscR to DNA was performed using DNase protection assay and showed that the IscR protected region covering the RNA polymerase-binding region (−35). The sequence upstream of P. aeruginosa iscR contains two IscR-binding motifs. The first AT rich region of each sites were substituted with CCC indicated that both binding sites were important for Isc-binding on the isc promoter. Overall data presented the molecular characterization of IscR-binding motifs in this pathogenic bacterium.

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P10-6

Roles of genes encoding glutathione biosynthesis against oxidative stress and virulence in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen in hospitalized patients. During infection, *P. aeruginosa* is encountered with reactive oxygen species (ROS) generated by host immune as a defense mechanism. Oxidative stress occurs when the bacterial cell expose to the ROS which causes oxidative damage to the cell. In order to survive under this condition, *P. aeruginosa* evolved both antioxidant enzymes and molecules to protect itself against ROS toxicity such as catalase, superoxide dismutase, and glutathione (GSH). GSH is involved in maintaining cellular homeostasis, metabolite conjugation, xenobiotic detoxification, antibiotic resistance, and stress response gene expression. GSH is synthesized by two steps catalyzed by γ-glutamylcysteine synthetase encoding *gshA* gene and glutathione synthetase encoding *gshB*. This work was investigating the role of *gshA* and *gshB* producing GSH against oxidative stress and virulent pathogenicity in *P. aeruginosa*. *gshA*, *gshB* and double mutants were constructed. These mutants showed susceptibility to methyl viologen and thiol depleting agent compared with the wild-type PAO1. These phenotypes could be complemented by functional *gshA* and *gshB* genes or GSH exogenously supplementation, indicating that the phenotypes of the *gsh* mutants arose from lacking of GSH. The *gsh* mutants had an attenuated virulence phenotype in fly *Drosophila melanogaster* model. Virulence factors including pyocyanin, pyoverdine, and cell motility were reduced in these *gsh* mutants These data indicated that *gshA* and *gshB* genes play important roles in oxidative stress protection and bacterial virulence in *P. aeruginosa*.

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P10-7

Effect of copper nanoparticles on nitrification in a soil–plant system

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Copper nanoparticles (NCu) are currently being used on many technological applications due to their antimicrobial activity. However, their increasing use raises concern about their toxic effect on the environment and specifically on geochemical cycles in soil. The main objective of this work was to evaluate the impact of NCu on nitrification in a soil–plant system. Two NCu doses (0.5 and 1.5 g kg^{-1}) were added to a soil–plant system previously established. Soil was sampled three times (1, 3 and 8 weeks) after NCu application and dehydrogenase (DHA) and urease activities were assessed. Copper fractionation and bioavailability were also measured, as well as microbial profiles of bacteria (16S) and *amoA* gene by DGGE. Additionally, the impact on soil nitrification kinetics was evaluated by maximum rate of nitrification (V_{max}). All treatments were set in triplicates and copper sulfate (CS) was used as reference as bulk form of metal. The results showed a dose-dependent effect of NCu on DHA and urease activity. A negative effect was observed on nitrification kinetic evidenced by a significant reduction ($p < 0.05$) on V_{max} value. Fractionation showed that NCu was predominately distributed in carbonates and Fe–Mn oxides and their bioavailability decreased over the time. DGGE profiles showed alterations in bacterial and *amoA* genes due to NCu independent of the evaluated dose. According to these results, it can be concluded that NCu in soil could be a risk to bacterial communities and nitrification process in a soil–plant system, that could lead to adverse biological effects.

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P10-8

Molecular mechanisms of thermotolerance and thermal adaptation in thermotolerant ethanologenic *Zymomonas mobilis*

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As global warming becomes more conspicuous, it is urgent to reduce carbon dioxide emissions and the use of biofuels is recommended. Because global demand for bioethanol is increasing, we should develop energy saving and efficient ethanol production technologies for the utilization of various biomass types.

High-temperature fermentation (HTF) is considered as one such technology, and its benefits such as reduction of cooling cost and water conservation in the fermentation process are expected, and consequently, the running cost is reduced. However, in the case of HTF, development of thermotolerant and efficient microorganisms is essential, and for stable fermentation, knowledge of thermotolerance is necessary.

In order to understand the molecular mechanism of thermotolerance of thermotolerant and ethanologenic *Zymomonas mobilis* TISTR 548, we identified genes involved in survival around a critical high temperature (CHT) by transposon mutagenesis [1]. These genes, called thermotolerant genes, were categorized into 9 groups related to fundamental cellular functions. These categories are similar to those for thermotolerant genes of intrinsically thermotolerant *Escherichia coli* [2] and thermotolerant *Acetobacter tropicalis* [3], suggesting that they share similar mechanisms for survival at a CHT. Moreover, to further improve thermotolerance, we performed thermal adaptation of two strains of *Z. mobilis* and identified mutations responsible for the adaptation. These experiments suggest that there is genomic capacity for thermal adaptation and its limit, and that there are several common molecular mechanisms.

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P10-9

Evolutionary engineering of *Cupriavidus necator* for improved utilization levulinic acid

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Polyhydroxyalkanoates (PHA) are bacterial polyesters that are considered as biodegradable and environmentally friendly alternatives to petrochemical plastics. The most common of them is poly-3-hydroxybutyrate (P3HB) which, although having material properties very close to polypropylene, lacks some qualities. Above all, it is very stiff and brittle. However, in the case of copolymers such as P3HB-co-3HV containing 3-hydroxyvalerate units (3HV), the most problems can be eliminated. The 3HV/3HB ratio significantly influences the different characteristics of the copolymer, such as melting temperature or impact strength. In order to produce the copolymer, it is necessary to add 3HV precursors which bacteria can incorporate into the copolymer structure. These are mostly expensive, which affects the already expensive PHA production. Levulinic acid (LA) is possible precursor, because it can be produced cost effectively and in high yield from renewable feedstocks. LA is a relatively cheap substrate and compares favorably with other possible precursors like propionic acid or valeric acid.

The aim of work was to use the concept of adaptive laboratory evolution to obtain strains capable of utilization LA. Adaptation tests were carried out with the *Cupriavidus necator* H16 strain. Higher LA concentrations have been shown to significantly reduce the growth of the selected strain. Adaptation tests led to an increase in the proportion of PHV in the copolymer produced.

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P11-1

Hot spot identification by ligand-protein surface interactions mapping – *in silico* study

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Haloalkane dehalogenases possess the ability to cleave carbon-halogen bonds in halogenated aliphatic compounds. They are becoming subject of many studies because of their potential use in bioremediation. Their active center is formed in a cavity hidden deep inside protein core. Before reaction takes place, substrates need to be transferred from surrounding solution to active site cavity. Previous experimental studies revealed an allosteric effect which suggest that despite substrate binding in active site, ligands can bind to the surface and participate in regulation of enzyme activity. Such findings have initiated our research concerning searching for surface amino acids which interact with substrates and controlling well-studied LinB dehalogenase activity.

We performed classical molecular dynamics simulations using Amber14 package to study spontaneous delivery of two different substrates: bromocyclohexane and 1,2-dibromoethane. The preliminary results show that we are able to capture the ligands entry phenomena. Furthermore, we are observing that particular sections of protein surface are able to trap and hold substrates prior to their entry to the active site. Detailed analysis yields information about the retentions of substrates in different compartments of protein surface, hence we constructed the surface-ligand contact map. It might shed a new light on importance of substrate transportation phenomena for selectivity and activity of LinB enzyme. The proposed approach can be used for identification of potential hot-spots for fine-tuning of an enzyme activity and selectivity.

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P11-2

Evolution of functionally important compartments of proteins – what can we learn from studying epoxide hydrolases?

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The variability of the residues in particular positions of a protein sequence highly depends on the protein region and its function. The surface amino acids evolve faster than buried residues. Active site residues and cavities are considered to be preserved during evolution of protein families, since they ensure an enzyme's activity

and selectivity. Despite these compartments, there is no research concerning the evolution of functionally important residues buried inside enzyme core. Growing evidence of the importance of transportation pathways linking the active site with protein surface raises the question of tunnels conservation. Soluble epoxide hydrolases (sEH) are a good case study for analysis of the evolutionary rate of tunnels. They are relatively small proteins, consist of an α/β fold core domain connected with a variable cap domain. The active site of sEH is located inside the protein core and is connected to the exterior by tunnel(s).

The CAVER 3.0 was used for tunnels identification in both crystal structures and all snapshots of molecular dynamics simulations. Using the R package BALCONY (Better ALignment CONsensus anal-Ysis), we compared the entropy values of the residues that make up tunnels and different compartment in four selected sEH, which represent animals, plants and bacteria. We discovered that the evolutionary rate of tunnel-lining residues depends on their position along the tunnel. We also provided a good approach for the selection of highly variable tunnel-lining residues as targets used for the fine-tuning of selected enzymes.

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P11-3

Virtual screening and bioassay of specific potential inhibitors for *Arabidopsis* group A protein phosphatases type 2C

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Arabidopsis group A protein phosphatases type 2C (PP2C) are important regulators and effectors of the abscisic acid pathway (ABA). The current knowledge about of the catalytic mechanism of PP2Cs inactivation facilitate development of group A PP2C inhibitors. In our approach we used modern bioinformatics and computational chemistry methods to identify small molecules with potential inhibition effect on PP2Cs group A. The crystal structure of the ABI1 PP2C with PYL1 receptor complex is used as model of binding site for inhibitor candidates in virtual screening (PDB code 3KDJ and 3NMN). This procedure allows to better characterize the binding effects and to consider the amino acids side chains dynamics in ligand binding. Over twenty millions chemical compounds from ZINC database were used for docking studies. Virtual screening procedure identified 325 chemical compounds as potential ABI1 inhibitors. The precision of the calculations was increased by quantum chemistry, free energy and molecular mechanics methods mainly to quantify the approximation of binding energy. According to chemical fingerprints selected molecules were clustered, ranked by scoring function and the binding energy was evaluated using quantum mechanical calculations. The activity of the top compounds in complex with PP2C group A members was analyzed in vitro. Resulting data allow identification of putative PP2C group A inhibitors that can be used in agriculture for control of ethylene evolution or broadly for crop protection against various stress conditions.

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P11-4

Water tracing as an alternative method for tunnels exploration in macromolecules

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Molecular dynamic simulations gave an insight into processes which occur in macromolecules. In some proteins active site is buried deep in the core to which access is enabled only by tunnels. The increasing awareness of tunnels role in enzymes with buried active site lead to development of several tools for cavities and pathways identification in macromolecules. The most recent facilitate information about the geometry of detected pathways along with their prolongation in time. Such approach, however, provides an approximation of tunnels, neglects physiochemical properties of amino acids which build tunnels, and therefore possesses certain limitations.

Simulated protein is immersed in water and thousands of single molecules are penetrating its core during a course of a molecular dynamic simulation. This phenomena was used to apply a different approach, in which molecules of solvent are traced in order to define the shape of cavities in proteins core. This technique, however, requires screening of positions of numerous single molecules along several thousand molecular dynamics steps. To facilitate analysis of the behaviour of water and other solvent molecules or ligands AQUA-DUCT was developed. AQUA-DUCT stands in for tools which search for tunnels, optimizing the process of water tracking and yielding clear results. It can be applied for analysis of macromolecules tunnels structure, properties and permeability, as well as accessibility of active site in proteins and ligands tracking.

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P11-5

MicroRNA–mRNA interaction network indicates a major role of MAPK pathway in fetal hemoglobin reactivation in beta-thalassemia

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Reactivation of fetal hemoglobin (HbF) in adult life is one of the most exciting therapeutic options to alleviate symptoms of beta-thalassemia. Researchers across the globe have been trying to identify the genetic and epigenetic mechanisms that lead to the regulation of HbF. Among the various known regulators, microRNAs (miRNAs) are believed to play crucial role in regulation of the gene expression. We investigated the possibility of identifying miRNA–mRNA regulatory networks using bioinformatics-based approach on datasets available in public resources. As a part of this study, we downloaded miRNA expression dataset (GSE93973) from NCBI Gene Expression Omnibus (GEO) and analyzed by comparing miRNA expression patterns in beta-thalassemia minor with high HbF subjects and normal healthy controls. Gene targets of differentially expressed miRNAs were identified using computational prediction and experimental validation as available from miR-

Walk2.0. The miRNA–mRNA interaction network was constructed using Cytoscape and functional enrichment analysis was performed using DAVID v6.8. A total of 20 differentially expressed miRNAs and 1990 miRNA–mRNA interaction pairs were identified. Additionally, hierarchal pattern of genes and miRNAs were identified using various topological measures like degree centrality, betweenness centrality and eigenvector centrality. Furthermore, hsa-miR-186-3p, hsa-miR-301b-3p and hsa-miR-125a-3p were identified as significant miRNAs based on number of target genes and centrality measures. Functional enrichment analysis revealed MAPK pathway as most enriched pathway with enrichment score 0.81. Therefore, these findings indicate towards major role of MAPK pathway in reactivating HbF expression. Future research should focus on exploring the prospects of using MAPK regulators of HbF reactivation.

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P11-6

Diversity of the genes involved in Algerian families with hearing loss identified by whole exome sequencing

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Hearing loss is a common sensory defect in humans. Autosomal recessive nonsyndromic hearing loss (ARNSHL) is the most common type and accounts for ~80% of cases of inherited hearing loss. Finding the responsible mutations via traditional methods in families with ARNSHL is difficult due to a high degree of genetic heterogeneity. Whole exome sequencing provides unprecedented opportunities to identify causative DNA variants in Mendelian disorders.

To identify the hidden mutations that cause ARNSHL we performed whole exome sequencing of 19 unrelated Algerian families with ARNSHL who were negative for mutations in GJB2 (the gene most frequently involved in ARNSHL in Mediterranean countries).

We identified the causative mutations in all the patients analyzed, either in the homozygous state (seventeen families) or in the compound heterozygous state (one family): (c.100C>T: p.(R34*)), (c.821C>T: p.(Pro 274Leu)) and (c.1534C>T: p (Arg512*)) in TMC1, (c.242G>A: p. (Arg81Gln)) in LRTOMT, (c.709C>T: p.(Arg237*)) and (c. 2122C>T: p. (Arg708*)) in OTOF, (c.1334T>G: p.(Leu445Trp)) and (c.2162C>T: p. (Thr721Met)) in SLC26A4, (c.518T>A: p. (Cys173Ser)) in LHFPL5, (c.5336T>C: p. (Leu1779Pro)) in MYO15A, (c.1807G>T: p.(Val603Phe)) in OTOA, (c.6080dup: p.(Asn2027Lys*9)) in PTPRQ, and (c.6017del: p.(Gly2006Alafs*13), (c.7188_7189ins14: p.(Val2397Leufs*2)) in GPR98.

Notably, 7 of these 14 mutations affecting 9 different genes had not been reported in other countries. We also identify an homozygous frame-shift mutation (p.Ser339Alafs*15) in EPS8L2, a new gene implicated in progressive deafness in human.

These results highlight the genetic heterogeneity of ARNSHL in Algerian families

Keywords: Algeria; Hearing loss; Genetic heterogeneity; Whole exome sequencing

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P11-7

Assessing coral stress responses using microarray

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The Kuroshio is a warm current, subject to rapid warming. In that regard, it warmed most rapidly in 1981–1998, when sea surface temperatures rose by 1.5 °C (0.9 °C/decade), almost 7 times the global rate. Because this current runs from tropical Philippines, through subtropical Taiwan, to the temperate region of Japan and Korea, it transfers heat from lower to higher latitudes. Because the Kuroshio Current is warming quickly, reef building corals in the region have rapidly expanded their range northward in response to warming temperatures. Furthermore, coral reefs of Taiwan and Japan were closely linked by the Kuroshio Current and inevitably, the coral population and its distribution in Korea could be affected by warming currents, similar to the situation in Japan and Taiwan. In this study, we chose Alveopora japonica which is a hard coral rapidly expanding the population around Jeju Island to construct microarray. The probes in microarray was based on the noble and known genes sequences from NGS technology. Approximately 60,000 genes from Alveopora japonica were used for microarray and we carried out temperature and pH stress experiment independently using microarray. We investigated the transcriptional changes in coral exposed to acidified seawater and also exposed to the high temperature and found several categories of functional genes responding to those stresses.

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P12-1

Synthesis and functional evaluation of nanoparticle conjugated antibiotics

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Objective: Nanoparticle conjugated antibiotics can be a promising means for withstanding drug resistance. The nanoparticle is supposed to increase antibiotic efficacy providing a synergistic effect. We focus here mainly on AgNP conjugated penicillin. Our primary goal is synthesizing silver nanoparticles and evaluating their antibacterial efficacy while co-administering with penicillin such as benzylpenicillin, ampicillin, amoxicillin, etc.

Methodology: Activity of nanoparticle largely depends on its size and shape. Evidence shows that smaller and spherical particles are more active than others. There are many ways of synthesizing nanoparticles. We have used chemical synthesis by reduction method as greater control over sizes can be achieved by this way. Characterization of nanoparticles has been done using UV–vis spectrometry and antibacterial efficacy has been checked using agar disc diffusion method against both gram classes of resistant bacteria.

Results and discussion: In case of reducing $AgNO_3$ with trisodium citrate or ascorbic acid, $NaBH_4$ color change indicates the formation of nanoparticles. UV–vis spectroscopic method has been used for further characterization of formed AgNPs. A sharp

peak appeared in the range of 300–400 nm indicates the formation of uniform nanoparticles while a broadening of the peak indicates that particles are polydispersed. Besides antibacterial activity of AgNPs has been investigated against resistant strains collected from different hospitals where no zone of inhibition has been found in presence and absence of antibiotics.

Conclusion: After several trials, the optimized method can be developed to synthesize silver nanoparticles of desired particle sizes. Once the desired particle size is obtained, characterization of the AgNP particles will be done by SEM/TEM. Conjugation with penicillin will be started as the second phase of the study.

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P12-2

Gold nanoparticle-based nanobiosensor for biomedical application

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Due to the desire to acquire an ameliorated quality of life and the phenomenon of population aging, the healthcare paradigm has been spontaneously shifting from diagnosis and treatment of a disease to prevention of a disease through the awareness of ailment. This has implemented a new lifestyle where individuals has the ability to better take care of themselves. Within these social context, there has been an increase in demand for an innovative healthcare system that involves biosensors which comprises of combined knowledge from the field of biotechnology and nanotechnology. Biosensor is a crucial factor that holds a key to dramatically improve our healthcare system. The importance of biosensor is supported through the recent increase in the investment and research development of biosensors with the technology from the field of nanotechnology. In order to successfully implant this novel technology, we need to develop a cost-effective biosensor with an ability of expeditious real-time diagnosis and monitoring of disease. In the case of nanobiosensor that uses metal nano-materials like gold and silver nanoparticle meets these standards mentioned above. Moreover, hypersensitive Nanobiosensor technology makes it possible to measure very low concentration of biomolecule, which enables early diagnosis to prevent aggravation of diseases. In this research, new type of Nanobiosensor platform using gold nanoparticle is suggested that is label-free, highly sensitive, quantitative detection possible. The suggested Nanobiosensor platform combines with different forms of gold nanoparticle or microfluidic device, enabling the production of multi scanning system, quantification of various biomaterials, and monitoring diverse biological phenomena.

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P12-3

Glutathione-responsive PEGylated GQDs as theranostic agents of HER2-positive breast cancer

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Graphene Quantum Dots (GQD) are attractive platform for diagnosis and as well as treatment of cancer due to its unique optical properties. In particular, targeted delivery of GQD exhibiting reduction-responsive properties is highly desirable. In this study, novel GQD-NPs were synthesized *via* EDC coupling reaction between the two precursors, DOX-SS-GQD and PEG-SS-HCT. Role of the first precursor was to maximize the drug loading capacity. The latter was introduced to improve half-life *in vivo* and to confer active targeting ability towards HER2-positive cancer. It is worthwhile to note that the GQD-NPs contain multi-cleavable disulfide linkages in between the junctions of the compartments for controlled release of the DOX. As intended, the drug release experiments, obtained from UV spectroscopy, demonstrated controlled release of the drug at the glutathione concentration relevant to the cancer cells. Furthermore, in vitro biological assessment, using SK-BR-3 (HER2-positive) and MDA-MB-231 (HER2-negative) cells as breast cancer models, confirms that they act specifically on the HER2-positive breast cancer cells. Intracellular trafficking through confocal laser scanning microscope shows not only the successful internalization of GQD-NPs in the sub-cellular region, but also, an enhanced cellular uptake with SK-BR-3. Furthermore, GQD-NPs displayed selective antitumor activity with SK-BR-3, suggesting intracellular release of the DOX. The GQD-NPs, with such selective antitumor activity, have potential as a theranostic agent for HER2-positive breast cancer cells.

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P12-4

Simple coating method by UV polymerization of caffeic acid with silver nanoparticles for implant applications

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Titanium has been largely used in implant manufacturing due to their excellent biocompatibility, great corrosion resistance, and mechanical properties. In this study, we present caffeic acid (CA) mediated immobilization of silver nanoparticles (AgNPs) on the surface of titanium materials as a simple method using UV irradiation to prevent inflammation of implants. CA is plant-derived phenolic compounds, rich in catechol and pyrogallol moieties, can form multifunctional coatings under the alkaline condition with UV irradiation which can trigger oxidative polymerization and deposition. Moreover, using CA can be a major component in the bone composition, causing decreases in inflammation by decelerating macrophage and osteoclast activity. Here, Surface characteristics and composition were investigated by using field emission scanning electron microscopy (FE-SEM), energy dispersive spectrometer (EDS), Fourier-transform infrared spectroscopy (FTIR), and atomic force microscopy (AFM). Antibacterial studies show that CA with AgNPs which we known as a material that has advantages of the excellent antibacterial could surprisingly enhance their antibacterial properties. The anti-inflammatory property was also evaluated with quantitative polymerase chain reaction assay (q-PCR), and cytotoxicity on fibroblast (NIH-3T3) cell lines were observed in vitro experiment. Therefore, our results suggest that the introduced approach can be considered as a potential method for therapeutic implant application.

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P12-5

Synthesis and characterization of cellulose nanocrystal from eucalyptus pulp

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Cellulose nanocrystal has received much attention as a promising wide range of research fields due to its compatibility with different media, and thermal stability. Acid hydrolysis is a common method used for extracting nanocrystal from biomass. Optimization of experimental parameters are required to obtain maximum yield and preserve nanocellulose morphology. In this research work, cellulose nanocrystal was prepared from eucalyptus pulp by H_2SO_4 hydrolysis. The effect of preparation parameters such as H_2SO_4 concentration (30, 40 and 50 wt%), hydrolysis time (30, 60 and 90 min) and hydrolysis temperature (60, 70 and 80 °C) on modified cellulose were determined. Physical and chemical properties of modified cellulose were investigated by X-ray diffraction, Scanning Electron Microscope (SEM), Fourier Transmission Infrared spectroscopy (FTIR) and thermogravimetric Analysis (TGA). Results show that an increase of H_2SO_4 concentration, time and temperature leads to lower percentage of crystallinity and reduction of crystal size. The percentage of crystallinity and crystal size were in range of 64.49–75.23% and 48.19–57.43 nm, respectively. The optimized condition was at 40 wt% of H_2SO_4 concentration 80 °C and 90 min that provide 64.49% of crystallinity and 48.19 nm of crystal size. It was also found that modified cellulose nanocrystal is more thermal stability than unmodified one. This evidence suggests the potential of this material in many applications including nanoscale reinforcing, rheology modifiers for drilling fluids in oil wells.

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P12-6

Nanobiocatalytic antifouling via quorum quenching

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Biofilms, occurring ubiquitously in aqueous environment, causes troubles such as membrane fouling, barnacle attachments on ship hulls, and even contamination of medical devices. One of the important mechanisms for biofilm formation is quorum sensing, which regulates bacterial physiology by small signaling molecules, called autoinducers, leading to the secretion of slimy extracellular polymeric matrices. Enzymatic inactivation of autoinducers using quorum quenching enzymes is a good candidate for environmentally-friendly antifouling. However, the poor stability of enzymes is a serious problem that can hamper the practical applications of enzymes for a long-term operation. Nanobiocatalysis, using nanostructured materials for enzyme immobilization, has gathered a growing attention due to its unprecedented successes in stabilizing the enzyme activity. This presentation will introduce the recent developments of nanobiocatalysis with a quorum-quenching acylase for membrane antifouling in wastewater treatment. Acylase was immobilized and stabilized in/on nanostructured materials via nanobiocatalytic approaches for their successful antifouling of membrane via quorum quenching. Several representative examples of nanobiocatalytic antifouling will be presented in detail.

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P12-7

Bioelectronic nose and tongue based on human receptor-carrying nanovesicles

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Human olfactory and taste systems were integrated with carbon nanotube platforms for the development of bioelectronic nose and tongue. Human olfactory and taste receptors were expressed on the surface of human embryonic kidney (HEK)-293 cells. Nanovesicles carrying human olfactory receptors (ORs) or taste receptors (TRs) were produced by treating the surface of HEK-293 cells with cytochalasin B. The nanovesicles, which generate olfactory/taste signals through a cAMP pathway, were integrated into single-walled carbon nanotubes field-effect transistors (SWNT-FETs). The nanovesicles and SWNT-FETs play roles in perceiving specific odorants or tastants, and in amplifying cellular signals, respectively. This system can be used for various applications including disease diagnosis.

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P12-8

Preparation of multi-walled carbon nanotubes film and immobilization of glucose oxidase for fabrication of enzyme electrode

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This study describes the enzyme electrode fabricated with multi-walled carbon nanotube (MWCNT) films for glucose-biofuel cells. The MWCNT film was prepared by vacuum filtration method, in which different amount of MWCNTs were dispersed in solvent with different concentration of dimethylformamide (DMF) and films were dried at different temperatures. Tensile strength and electro-conductivity of the prepared MWCNT films were measured and their surface properties were characterized by SEM, TGA, Raman spectroscopy. Glucose oxidase (GOD) was immobilized on the film by layer-by-layer and gold nanoparticles (GNPs) were also deposited between the GOD layers to enhance their electron transfer properties of the electrode. The electrochemical performances of GOD-film electrodes were evaluated using cyclic voltammetry and impedance analysis. Immobilization of glucose oxidase (GOD) on MWCNT films increased their electron transfer resistance, which could be mitigated by introducing GNP layers between GOD layers. The highest current was generated by films with sequential three layers of immobilized GOD and GNPs. The assembled biofuel cells comprised the GOD-immobilized films (anode), laccase-immobilized gold electrode (cathode), and reference electrode. The electrodes were placed in 30 mM of glucose solution. The maximum power density was 1.02 ± 0.03 μW/cm^2 at 0.51 ± 0.01 V of cell voltage.

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P12-9

Extraction methods to detect biopolymer-based nanoparticles synthesized by recombinant strains of *Saccharomyces cerevisiae*

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At recent years eco-friendly nano-biopolymers, such as polyhydroxyalkanoates-based nanoparticles (PHA-NPs) play an important role at nano-biosciences due to their novel applications, resulting of its high ratio of superficial area/volume. However, the difficulties to control the size of nanoparticles are still a limitation for their production. We report the development of two recombinant strains of Saccharomyces cerevisiae to synthesize PHA-NPs by bottom-up process, expressing chimeric proteins onto vesicles, an internal yeast organelle. The expression allows these strains to polymerize available (R)-HA-CoA monomers from cytosol at the vesicle surface, synthesizing PHA-NPs. The natural transportation process of vesicles from Golgi complex to membrane, facilitate the secretion of already synthesized PHA-NPs across the membrane, towards the culture medium.

In this work, flask fermentations were performed, collecting the cells and medium for further analysis. We uses techniques, such as scanning electron microscopy, dynamic light scattering, 3D cross-correlation light scattering and Fluorescence assays with Nile red stain, to detect intracellular and extracellular PHA-NPs synthesized by recombinant yeast strains. Also, gas chromatographic measurement of biologically produced PHA-Nps (intracellular and extracellular) using various extraction and purification protocols, are reported.

Therefore, the study of this novel bottom-up model for synthesizing NPs provides a new range of opportunities to produce organic NPs.

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P12-10

Affibody-functionalized gold nanoparticle-based ELISA for dengue virus NS1 antigen

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Infection with dengue virus (DENV) is a serious health issue that causes severe dengue fever and occasionally lethal complications, such as dengue hemorrhagic fever. Due to the absence of a licensed vaccine or antiviral drug, rapid and sensitive DENV detection is important to reduce morbidity and mortality. Here, we developed a highly sensitive enhanced enzyme-linked immunosorbent assay (ELISA) for dengue NS1 using affibody-functionalized gold nanoparticles (AuNPs). First, we screened NS1 antigen-specific affibody molecules ($Z_{NS1}12$, $Z_{NS1}16$, and $Z_{NS1}46$) from the affibody phage library. The affibodies were then expressed and purified from Escherichia coli. Among them, the $Z_{NS1}12$ affibody showed the highest equilibrium binding constant (Kd) of 1 μM. This affibody was functionalized on AuNPs measuring 20 nm in diameter. The developed anti-NS1 affibody-functionalized AuNPs (($Z_{NS1}12$)2-AuNP) were used as carriers to achieve amplification of the signal. ($Z_{NS1}12$)2-AuNP showed good properties, such easy synthesis, high number of affibodies conjugation on AuNPs, and excellent stability under harsh conditions with high salt concentrations and temperature. In addition, this nanoparticle-based enhanced ELISA resulted in a 14.2-fold signal amplification performance for dengue NS1 detection in comparison with conventional ELISAs. This novel and sensitive method using ($Z_{NS1}12$)2-AuNP may have applications in the detection of DENV in infected patients at an early stage and for the detection of other pathogens in clinical diagnostics.

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P12-11

Proteolytic enzyme loaded chitosan nanogel for efficient treatment of spinal disc herniation

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The chymopapain as a proteolytic enzyme has been used in clinics for the treatment of spinal disc herniation. However, the fast and wide diffusion and spreading of the enzyme from the injection site limit its effect and could cause severe side effects. Bare and chitosan conjugated nanogel were prepared by photo-crosslinkin. Chymopapain was efficiently loaded into the nanogel with an encapsulation efficiency of over 95% in all the nanogel. In vitro release studies in physiological conditions (pH 7.4, 37 °C) showed a sustained release of the enzyme from all the nanogel but release from chitosan nanogel (~16 days) was slower than that from the bare nanogel (~12 days). Chymopapain delivered by nanogel remained more localized at the injection site in the nucleus pulposus of ex vivo porcine discs, compared to free chymopapain. Chitosan functionalized nanogel showed longer retention and slower diffusion of the enzyme compared to bare nanogel.

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P12-12

Hybrid nanocomposite of iron oxide nanoparticle/chitosan nanocarrier for efficient tumor targeting and imaging

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For efficient tumor targeting and imaging, we developed organic/inorganic hybrid nanocomposite composed of an iron oxide nanoparticle (IONP)-loaded chitosan nanocarrier. The different loading contents of IONP was introduced to modulate the mechanical properties of the system, and compared the characteristics of tumor targeting and imaging in terms of loading contents of IONP. The chitosan nanocarrier with useful properties such as long blood circulation, good tumor targeting, and easy loading of macromolecules was used. IONPs were efficiently encapsulated into the nanocarrier (high loading efficiency over 95%). Overall, very good tumor targeting and accumulation of IONP were achieved by using the chitosan nanocarrier, thus, this could serve as an enhanced MRI contrast agent. On the other hand, the different loading contents of IONP clearly affected the accumulations in the tumor and the liver, thus the quality of MR imaging was also affected by the loading contents of IONP in the nanocarrier. A high loading content (40 wt.%) with rigid properties significantly reduced the accumulation and MR imaging signal in the tumor and increased the liver uptake.

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P12-13

New three-component delivery system for beta-carotene

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β-Carotene is a natural lipophilic compound belonging to the carotenoid family. It is a precursor of vitamin A and exhibits antioxidant activity. However, the application of β-carotene in functional foods and dietary supplements is currently limited due to its very low water solubility and air-, light- and temperature sensitivity.

The new three-component system for β-carotene delivery was developed. Inclusion complexes of β-carotene with 2-hydroxypropyl-β-cyclodextrin were prepared by co-precipitation method. They were characterized by FT-IR and NMR spectroscopy, and the complexation efficiency was determined by HPLC. Further, the prepared water-soluble inclusion compounds were additionally complexated with pectin, and β-carotene-2-hydroxypropyl-β-cyclodextrin-pectin nanoparticles were obtained. For particles formation, pectins with different degree of esterification were used. The interaction of particle components was demonstrated by FT-IR spectroscopy. The size of particles was characterized by DLS method. Now, chemical stability of β-carotene encapsulated in prepared nanoparticles is analysed.

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P12-14

High pulsed field activation of magnetic nisin-loaded nanoparticles for antimicrobial efficacy

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Consumption of food, which is contaminated by pathogenic bacteria represents a serious threat for public health. Therefore, the applied research of antimicrobials, alternative or combinational methods for the biocontrol and sensitization of microorganisms is in constant focus. One of such prominent bacteriocins is nisin, an oligopeptide produced by certain strains of Lactococcus. It exhibits a wide spectrum of antimicrobial activity, while commonly being inefficient against Gram-negative bacteria. In this work, we present a novel antimicrobial methodology using targeted magnetic nisin-loaded nano-carriers (iron oxide nanoparticles capped with either citric, ascorbic or gallic acids), which are activated by high pulsed electric and electromagnetic fields. Gram-positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* were employed as model targets. We have demonstrated that it is possible indeed to increase the antimicrobial efficiency of nisin by using different encapsulation methods to improve the stability and binding of the structure to magnetic nano-carriers, while the nisin-resistance can be overcome by controlled poration of the cell membrane in the pulsed electric field. The pulsed electromagnetic field methodology can be further used to increase the treatment efficiency. As a result, we are first to present a proof of concept of novel antimicrobial methodology using magnetic nisin-loaded nanoparticles, which are activated by combination of electric and high pulsed electromagnetic fields. The results of our work are promising for the development of new methods for treatment of the drug-resistant foodborne pathogens to minimize the risks of invasive infections.

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P13-1

Isolation and characterisation of hydroxyproline-rich glycoproteins from green macroalgae

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Hydroxyproline-rich glycoproteins (HRGPs) represent a complex group of cell wall proteins ubiquitous to higher plants with various form and function. HRGPs are often categorized into i) non-glycosylated or minimally glycosylated proline-rich proteins, ii) moderately glycosylated extensins and iii) highly glycosylated arabinogalactan-proteins. However, with increasing data available it is obvious that this family comprises a continuum of molecules, since a substantial number of chimeric and hybrid HRGPs was described. Despite their relatively minor occurrence in primary cell wall compared to polysaccharides, HRGPs play an essential role in cell wall architecture, plant development, embryogenesis, signaling and defense against biotic and abiotic stress. Unfortunately, most of the known data are based on research in land plants and little is known about their occurrence, structure and function in algae. The biggest issue is missing sequenced genomes of most algal species. Therefore monoclonal antibodies developed against HRGPs, together with Yariv reagents, which react positively with arabinogalactan-proteins, are main tools to study these proteins in algae. The objectives of this study are to extract and purify HRGPs (mainly arabinogalactan-proteins and extensins) from Ulva lactuca, characterize them and test their influence on stress responses in land plants. Presence of HRGPs in Ulva lactuca was confirmed by immunohistochemistry experiments as well as Yariv reagents. Extraction protocol was optimized, and according to Western blots, extracts contain at least three HRGPs. Purification by ion exchange and size exclusion chromatography was tested and will be further improved.

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P13-2

Solamargine and solasonine production by plant parts *Solanum incanum* from Oman and their biological activity

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Traditionally important medicinal plant *Solanum incanum* is a rich source of cytotoxic glycoalkaloids (GAs) such as solamargine and solasonine. As a potential source of compounds for the synthesis of steroids, study of the content of these glycoalkaloids during developmental stages of plant is worthy. Quantitative estimation of solasonine and solamargine content using optimized isolation process and newly developed and validated HPTLC method in different parts of *S. incanum* plants at different developmental stages and comparing changes in the whole-plant glycoalkaloids profile during development of plants grown in Oman was carried

out. Solamargine and solasonine produced well separated compact bands at Rf values 0.26 and 0.14, respectively on silica gel HPTLC plate using chloroform: methanol: 5% ammonia (7:3:0.5, v/v/v) after visualization using anisaldehyde sulphuric acid reagent. Chromatograms scanned at 530 nm wavelength and method was found linear ($r^2 \geq 0.9962$) for 50–2000 ng/spot for both drugs. The validated method was used for analysis of glycoalkaloids in small, young, immature and mature leaves as well as stem and root parts up to the 40th week of plants' growth, showing rich concentration of glycoalkaloids with variation at different stages of development. Hence, highlighting the importance of developmental stages of particular organ and the overall age of the plant when harvesting for these GAs from *S. incanum* plants.

Anticancer activity of these glycoalkaloids has been studied; Solamargine was capable of inhibiting proliferation of melanoma cancer cell lines at a concentration of 10 μM but not the benign melanoma and normal cells.

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P13-3

Poplar calcium-dependent protein kinase CDPK5 regulates drought tolerance through Ca^{2+}-mediated ion homeostasis

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Forests, mostly made of tree species, are one of the main cover types in land ecosystems. *Populus euphratica*, the only tall tree species in Taklimakan desert, is an ideal system to study abiotic stress in woody plants. Here we show that PeCDPK5, a calcium-dependent protein kinase, plays a critical role in *P. euphratica* response to drought stress. PeCDPK5 was up-regulated by dehydration, osmotic and drought stress treatments, and showing multiple subcellular localization. Overexpression of PeCDPK5 strength stomatal closure and reduce waster loss in leaves, then enhance drought tolerance in *Arabidopsis thaliana*, as well as in poplar 84 K (*Populus alba* × *Populus glandulosa*). In roots, non-invasive micro-test technique (NMT) results showed that PeCDPK5 significantly promoted the influx of Ca^{2+} and lead to the decreased efflux of K^+. Meanwhile, PeTPK1 (*P. euphratica* two pore K^+ channel 1) located at tonoplast, was identified as a PeCDPK5-interacting protein, confirmed by yeast two-hybrid and bimolecular fluorescence complementation (BIFC) assays. PeCDPK5 can promote the expression of PeTPK1 and reduces K^+ exclusion under hypertonic environment. In general, these results indicated that PeCDPK5 functions in regulating drought tolerance through promoting stomatal closure in leaves and mediating ion homeostasis in roots.

Keywords: *Populus euphratica*; CDPK5; Drought stress; PeTPK1

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P13-4

Physiological screening of wheat genotypes grown under boron stress

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Boron toxicity is one of the critical abiotic stresses influencing agricultural growth and crop yield. Many dry regions of the world including Central Anatolian region of Turkey suffer from Boron toxicity caused by the extreme boron levels in the irrigation water and soil. These regions become de-limited to boron tolerant crops and finally become inappropriate for agriculture. In most of the regions, Boron toxicity is often accompanied with high salinity in the soil. Thus, it can be beneficial to have both salt and boron tolerant genes in same genotypes. However, the interaction of boron toxicity tolerant and salt tolerant genes is quite unclear. Marker assisted selection along with classical breeding has played an important role in the expansion of wheat varieties under stress conditions. Aiming this, we screened wheat genotypes from different origins to estimate their boron tolerance levels so that salt tolerance genes can be efficiently transferred in potential ones; and common boron and salt tolerant germplasm can be developed. Growth parameters measurement, MDA and proline estimation was conducted for the wheat genotypes grown under B deficient and B toxic conditions. Based on physiological results, different genotypes showed high boron tolerance levels, which were further selected for our back cross breeding program. Finally, we aim to develop wheat progeny combinations with both boron and salt tolerance genes that can be efficiently grown in regions suffering from combined boron and salinity stress conditions.

Keywords: Backcross; Boron toxicity; MAS; Molecular breeding; Soil salinity

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P13-5

Obtention of functional leaf protein concentrate (F-LPC) based on Ereky-process: historical survey and preliminary results

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According to the historical sources the pioneeer of Green Biorefinery concept was Károly Ereky, the father of the term 'biotechnolgy' in Hungary and England between 1917 and 1938. The Ereky-process is based on wet fractionation of grasses, alfalfa and other valuable freshly harvested green biomass. Ereky's Green Mill process and machines were patented in five countries. The concept is based on three distinct technological pillars. One of the main features was an improved green pulping technology to obtain biological valuable freshly fractionated 'green milk' rich in amino-acids, vitamines and other biologically active substances and easily digestible protein concentrate, called 'plasma preserve' to feed monogastric animals (1917–1939). The other purpose of the Ereky-process was the production of protein-enriched functional food additives (1926–1939). In collaboration with Béla Dorner, the process' third area was the establishment of new artificial silk production technology based on protein precipitate of green plant juices (1937–1939). After the Second World War Ereky's pioneering researches have been forgotten, and his methods were further developed by N.W. Pirie in England. The goal of our present work is producing functional leaf protein concentrate (F-LPC) based on the Ereky-process combined with the latest knowledges. Our research carried out with alfalfa (Proteomill Project, Tedej Ltd., University of Debrecen, BRC HAS, NF Mikrotech Ltd.) confirms that the mixture of fractionated plant juice and carriers to obtain fortified green pastry (F-APC) is possible and, after further development, this process seems to be a promising new opportunity for the Green Biorefinery industry.

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P13-6

Somatic embryogenesis of sweet pearl millet (*Pennisetum glaucum*), a rich source of cellulosic biomass

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Sweet pearl millet (*Pennisetum glaucum*, 2*n* = 14), an annual forage crop cultivated predominantly in semi-arid tropics throughout the world. In addition to nutritional importance, this crop is a very good source of cellulosic biomass, and being used as an alternative source of fossil fuels i.e. biofuel. Utilization of cellulosic biomass is been stagnant due to the presence of complex crosslinking with lignin. To reduce the quantity of lignin, the genetic engineering is a cost-effective way compared to other conventional process. As the in-vitro regeneration is a backbone of genetic engineering, so in this present study we have established an effective regeneration protocol of sweet pearl millet via somatic embryogenesis. The MS basal media supplemented with varying concentration of 2,4-D alone and in combination with varying concentration Kinetin (KIN) was applied to get embryogenic callous. In the second phage, well grown embryogenic callous were subjected to the medium supplemented with BAP alone or in a combination with KIN for somatic embryogenesis. Elevated embryogenic callous were observed in the media supplemented with 2,4-D and KIN with 96.66% callous induction efficiency. About 10 shoots/callous were obtained when callous were cultured in the medium fortified with BAP and KIN (regeneration efficiency = 76%). Furthermore, 100% rooting was observed in 1/2 MS basal medium. The regenerated plants not showed any phenotypic abnormalities in the course of hardening and outdoor growth. Described procedure is efficient and highly reproducible; therefore it may be useful for genetic manipulation of said plant.

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P13-7

Jerusalem artichoke (*Helianthus tuberosus L.*) green biomass as a potencial protein source

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Considering the growing demand for food and feed poses along with the effect of climate change, the diversity of genetic resources (species, varieties, breeds) should be a re-evaluated aspect of sustainable agricultural production. From the XX century a few major staple crop species became such as maize, wheat, rice, soy, rape. At the same, number of varieties has also been decreased based on FAO survey. The decline in agrobiodiversity has negative effect not only crop safety, but also on abiotic and biotic stresses.

Jerusalem artichoke can be one of the species that used to enhance the repertoire of agricultural crop production, even today is underutilized. Among several advantages it is more tolerant to harsher conditions than most commercial crops, resistant to most pests and disease, grows on most soils with little added fertilizer and multipurpose plant.

At present, Jerusalem artichoke is primarily cultivated for inulin content of tubers. However, during the vegetation period it develops a high amount of green biomass, which can be harvested two or three times. Although most of animals don't prefer the green leaves of Jerusalem artichoke, fractionated green biomass with high amount of protein and other phytochemicals can be valuable product for animal husbandry purposes.

In accordance with the above the aim of present study was to produce and evaluate leaf protein concentrate, brown juice and green fiber of green biomass of Jerusalem artichoke ecotypes using biotechnological tools.

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P13-8

Fructan and glucan-mediated priming in banana

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Fructans and glucans are naturally occurring polysaccharides in plants and can be synthesized by certain microorganisms. Generally, plant fructans are known as the main storage carbohydrates and have been studied as immunomodulators. However their occurrence has also been linked to abiotic stress resistance (Versluys et al., 2018). Potentially, fructans and fructooligosaccharides can induce immune responses and even enhance them by modulating gene expression profiles of various metabolites via signal transduction cascades thus priming plant defense mechanism.

Bananas (*Musa* spp.), one of the most important staple foods in the world, are natural fructan accumulators and highly vulnerable to drought. Genome level studies highlighted the significant difference in the regulation of carbohydrate metabolism between drought-resistant and non-resistant banana varieties suggesting the role of sugars in stress tolerance mechanisms (Muthusamy et al., 2016).

To explore the functional roles of glucans and fructans in plant defense, several plant and microbial-derived fructans of a different degree of polymerization and oat-derived β-glucans were tested by leaf and cigar priming on 3-month-old banana plants subjected to 5 weeks of water deprivation under greenhouse conditions. Leaf tissues were collected when the soil pF value was equal or higher than 2.8. Based on leaf area, cigar-primed plants showed lower stress levels with more uniform growth profile. Further research on quantification of internal sugar levels, stress-related gene expression patterns and enzymatic activities involved in sugar metabolism are expected to provide a good overview of banana stress responses under drought and the functional roles of fructans and glucans as immune response modifiers.

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P13-9

Heat shock protein 90 interplay with YODA signalling pathway modulates SPCH activity to regulate stomata development

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The key element of plant adaptive mechanisms for optimal water management and photosynthetic efficiency is stomatal development, which is controlled by SPEECHLESS (SPCH), MUTE

and FAMA, the major basic-helix-loop-helix (bHLH) transcription factors (TFs). Mitogen activated protein kinase (MAPK)-mediated transcriptional remodelling is crucial for the stomatal lineage specification where YODA is the upstream regulator of MPK3/6 kinases which regulate SPCH. HSP90 is an evolutionary conserved molecular chaperone that modulates a broad range of signalling pathways via interactions with a complex network of proteins. We monitored changes in stomatal patterning, distribution, and morphology through physical and genetic interactions between HSP90 and YODA. We show that HSP90 function modulates the transcript levels and protein abundances of downstream signalling targets, such as *SPCH*, *MUTE* and *FAMA*. Our data reveal that HSP90 and YODA signalling interplay control the mRNA levels of *SPCH*, *MUTE* and *FAMA*. We furthermore analysed SPCH protein levels in single and double *hsp90* and *yda* mutant genetic backgrounds. HSP90 depletion led to accumulation of SPCH, while it restored SPCH to wild-type levels in *yda*. SPCH-mediated transcriptional activity after HSP90 inhibition was monitored through the expression of SPCH transcriptional targets including *TMM*, *EPF2*, *POLAR* and *BASL*. HSP90 inhibition induced SPCH transcriptional activity, as key regulators of cell divisions *POLAR* and *BASL* and also negative feedback regulators *TMM*, *EPF2* were upregulated. Our findings establish HSP90 as a key regulator of stomatal cell development as HSP90 activity is crucial for cell fate determination modulating the transcriptional circuitry that shapes the physiological outcome.

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P13-10

Life cell imaging by means of structured illumination microscopy

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For advancing the characterization of intracellular organisation and dynamic processes of plant cell, it is essential to master techniques enabling detailed localization and co-localization studies of various proteins of interest. For this purpose, several super-resolution techniques have been employed in plant biology in recent years.

These super-resolution techniques circumvent or surpass the limitations of classical optical microscopy. Among other strategies, one way for overcoming the limits predicted by Abbe's equation is to illuminate the sample by patterned light. This approach is employed in structured illumination microscopy (SIM).

The platforms supporting SIM are capable of illumination of the sample by patterned light and subsequent reconstruction of image from raw data, which comes in form of Moiré patterns. Said platforms are able to reach practical resolution of image that is twice as good as the theoretical limit of classical optical microscopy. Despite other super-resolution techniques, such as stimulated emission depletion microscopy (STED) or photoactivation localization microscopy (PALM), reaching even higher resolution, the SIM is advantageous for life cell imaging for its unrestricted use of fluorophores and relative speed.

Thus, SIM is a convenient method for resolving organelles and various compartments and to follow dynamic reorganization of subcellular structures in living samples. Here, we are presenting the current application possibilities in employing SIM for life imaging of organization and dynamics of plant subcellular structures.

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P13-11

An overview of plant cell superresolution imaging

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Superresolution methods have been established over the past 20 years and represent an escape from the severe diffraction limitations of classical far-field microscopy. Their implementation in plant cell research has been slow, but steady and up to now there is a considerable amount of publications using structured illumination microscopy (SIM), photoactivation localization microscopy (PALM), stochastic optical reconstruction (STORM) and stimulated emission depletion microscopy (STED). Our presentation will provide an overview of these methods for the imaging of fixed and living plant samples. We will present the localization of microtubules, microtubule associated protein 65-2 and 65-3, end binding protein 1c (EB1c) and mitogen activated protein kinase 6. These proteins were fused with either conventional fluorescent proteins such as eGFP, or with photoswitchable or photoconvertible proteins such as DRONPA and mEos3.2. We are going to show localization of the above proteins by means of SIM, PALM/STORM and STED and were applicable we will compare living with fixed and immunolabeled probes.

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P13-12

Functional relationship between mitotic microtubules and phospholipase D alpha 1

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Phospholipase D alpha 1 (PLDα1) and its product phosphatidic acid (PA) play crucial roles in a variety of cellular and physiological processes including cytoskeletal organization and remodelling, regulation of stomatal closure and opening, as well as biotic and abiotic stress signalling. In this study, we showed association of PLDα1-YFP with mitotic microtubule arrays using immunofluorescence colocalization of PLDα1-YFP and microtubules in root meristem cells of complemented *pldα1-1* mutant seedlings expressing *proPLDα1::PLDα1:YFP* construct. Homogeneous cytoplasmic distribution of chimeric PLDα1-YFP protein was observed in non-dividing interphase and pre-mitotic cells. On the other hand, an accumulation as well as partial association of PLDα1-YFP with mitotic microtubules (preprophase band, spindle, early and late phragmoplast) was observed in mitotic root cells. Detailed observations revealed predominant association of PLDα1-YFP with leading edge of the phragmoplast. Moreover, PLDα1-YFP accumulated in developing cell plate during cytokinesis.

Super-resolution structural illumination microscopy (SIM) was used for clarification of functional relationship between PLDα1, mitotic microtubules and clathrin. Association of PLDα1-YFP with clathrin-coated vesicles located between cortical microtubules was observed using SIM. These results support a possible mechanism of interactions between clathrin-dependent endocytosis and microtubules, through possible stabilization and molecular linker function of the PLDα1.

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P13-13

Elimination of odontoglossum ringspot virus from orchids by novel antiviral protein produced by *Streptomyces*

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Orchids are economically important ornamental flowering plants belong to the family orchidaceae which are vulnerable to several diseases caused by the most prevalent odontoglossum ringspot virus (ORSV) in an orchid plant. In this study, the actinomycete was isolated from sediment soil and identified as *Streptomyces laurentii* based on the colony morphology, 16S rDNA sequencing by BLASTN and phylogenetic tree analysis. *Streptomyces* was cultured in a soybean meal mediums at different day's intervals and analyzed for the protease activity in skim milk casein agar. The best protease activity was observed at 21 days after cultivation. The crude enzyme was performed in Zymogram NATIVE-PAGE and SDS-PAGE to characterized the antiviral activity of the protein. Indirect ELISA assay was employed to confirm the antiviral activity of the target protein against ORSV infected orchid plant. The active fragment was observed at the band appeared around 47 kDa in the SDS-PAGE. This band is excised from the gel and subjected to in-gel trypsin digestion and analyzed by MALTI-TOF Mass Spectrometry. The resulting peptide fragments score matched with Elongation factor-Tu (EF-Tu) of *Serratia marcescens*. Furthermore, the culture filtrate of Streptomyces was precipitated by 60% $(NH4)_2SO_4$ and further purified by DEAE ion-exchange chromatography column. The purified sample still retain the remarkable antiviral activity against ORSV. In conclusion, the isolated Streptomyces culture has shown to be effective in the eradication of ORSV from infected orchid, and thus has the potential to be a good biocontrol agent for eliminate the virus contaminated orchid.

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P13-14

Mapping the floral volatile timeline of a summer and a winter-blooming Jasminum species for effective use in essential oil industry

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Jasmine flowers are in great demand worldwide owing to their unique fragrance. Floral extracts of jasmines are utilized commercially in cosmetic and beverage industries. Since the flowers are short-lived, knowing the temporal variations in the composition of floral scent volatiles, comprising of emitted, free endogenous and glycosyl-linked volatile compounds, is essential for obtaining an optimal yield of scent-associated compounds. In the present study, a targeted time-course analysis of the floral scent volatiles from two *Jasminum* species, viz. *J. auriculatum* (summer-blooming) and *J. multiflorum* (winter-blooming), has been elucidated. The matrix-solvent combination of Porapak Q (80/100) with dichloromethane (DCM) was found to be the most suitable amongst four different matrices and solvent system tested for emitted volatile profiling. The volatile emission in *J. auriculatum* flowers exhibited nocturnal maxima pattern. Flowers of *J. multiflorum* emitted its highest concentration at noon. In case of endogenous volatile compounds, it was observed that the floral volatiles with high molecular weight tend to retain in the floral tissue unlike the lower molecular weight volatiles from bud to senescence. Enzymatic hydrolysis of floral extract of both these species revealed that several volatile compounds (mostly alcohols) are biosynthesized and stored in flower tissues as water-soluble glycosides, and the concentrations were higher at late bud stage. The practical utility of this nature-inspired biological study would be an assistance to florist trade for ascertaining the precise plucking time of flowers intended for harnessing essential oils with specific volatiles in jasmines.

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P13-15

Impact of light-emitting diodes (LEDs) on the growth and physiological status of spinach (*Spinacia oleracea* L.) seedlings

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Implementation of LED (light-emitting diodes) lighting in plant-production systems is gaining popularity due to its versatility and energy efficiency as compared to conventional artificial light sources. In the present study spinach (*Spinacia oleracea* L.) seedlings were raised under blue (470 nm) and red (630 nm) LEDs via panels providing monochromatic and combined (blue + red, 1:3, 1:1, 3:1) spectral treatments, with fluorescent lamps (FLs) as control. Seedling growth was assessed in terms of height, leaf area and fresh weight, specific leaf area (SLA), number of leaves per seedling and canopy cover after 28 days. Chlorophyll, carotenoid, and total phenolic contents, non-enzymatic antioxidant activity (DPPH (2,2-diphenyl-1-picrylhydrazyl) and superoxide radical scavenging activities) and oxidative stress (H_2O_2 content, lipid peroxidation, electrolyte leakage) were recorded concomitantly to assess the impact of LEDs on the physiological status of the seedlings. Seedlings grown under blue + red LEDs showed better growth and higher chlorophyll content as compared to the monochromatic LED treatments. However, the total carotenoid content was more under high proportions of red LEDs. Total phenolic content was significantly increased under LEDs as compared to FL. DPPH and superoxide radical scavenging activities also exhibited significant increase in LED treated seedlings. Oxidative stress was lower in seedlings raised under combinations of blue and red LEDs as compared to the control. In conclusion, the present work demonstrates that manipulation of ambient light conditions by means of LEDs may be implemented for improving the quantitative yield and qualitative attributes of leafy vegetables being cultivated under artificial lighting.

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P13-16

Gamma irradiation impact on growth and physiology of *Euryale ferox*: an underutilized aquatic food crop

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The impact of gamma irradiation on growth and physiology of *Euryale ferox* was described in the present investigation. *E. ferox* is an underutilized aquatic food crop which grows in shallow water bodies in lower Assam regions and north Bihar of India. The seeds of *E. ferox* were irradiated with different doses of gamma irradiation ranging from 0 to 500 Gy. It was observed that the germination and survival percentage was inhibited by increasing the irradiation dose. However, plant developed from seed exposed to irradiation dose beyond 100 Gy did not survive more than one month. Further growth parameters (Leaf size and number, number of thorns, root number and length, and number of flower and seeds) were also compared with respect to non-irradiated plants. Physiological parameters viz chlorophyll a, chlorophyll b, total chlorophyll, photosynthetic rate, transpiration rate, stomatal conductance, and Intracellular CO_2 content was higher in irradiation population of *E. ferox*. Superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities were observed high in irradiated population of *E. ferox*. The proline and glycine betaine content enhanced with increasing the irradiation dose. The present investigation explores the potential use of gamma-rays in genetic improvement of *E. ferox* and improves understanding

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P14-1

Acceleration of wound healing induced by hyaluronic acid

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Hyaluronic acid (HA), one of the main component of extracellular matrix, is considered to be functional in wound healing. It has been reported that HA with high molecular weight (MW) would be degraded into low-MW fragments on the process of wound healing. However, it is not clear about the effect of molecular size on their bioactivities for wound healing. In this study, the effect of HAs with different MW on wound healing processes was investigated, especially focused on the epidermal cells.

Proliferation of HaCaT cells was assessed by WST assay. Migration ability was assessed by wound scratch assay. In order to examine the effect of HA addition on wound healing events *in vivo*, epidermal wounds were made to the back of hairless mice using a biopsy punch. Then, the wound area was measured at definite time interval.

Proliferative activity of HaCaT cells was enhanced by addition of high-MW HA in its dose- and MW-dependent manners. Almost the same results were obtained in its migration activities assessed by wound scratch assay. The HA's MW, which is the most effective in our studies described above, was included in the range of HA's MW frequently observed in the surrounding area of the epidermal cell in healthy body. Furthermore, the enhanced wound healing processes by addition of HA with high-MW was confirmed by *in vivo* study. From these results, it was suggested that MW and concentration of HA exert or are as important determinants in the process of wound healing.

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P14-2

Synthesis and antioxidant activities of sulfated polymannuronic acid (PMS) and sulfated polyguluronic acid (PGS)

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Alginate is comprised of Polymannuronic acid (PM) and polyguluronic acid (PG) with different ratios depending on its source. The aim of the research was to investigate the effect of the sulfate content of PM and PG on their antioxidant activities. In this study, separated PM and PG were obtain by chemical hydrolyzation and subsequent pH fractionation. Chlorosulfonic acid/formamide were employed for sulfation of the PM and PG resulting the PMS and PGS having a degree of sulfate substitution at 0.66 and 0.53, respectively. Among of the non- and sulfated PM and PG, the PGS contributed the highest percentage of superoxide radical scavenging (29%) which was accounted for about half of ascorbic acid capability. This was also true for the reducing power assay that PGS yielded the highest ability in donating hydrogen and electrons to free radicals in the examination system. The PGS and PMS showed approximately twice the ability in hydroxyl scavenging compared to the non-sulfated polymers. On the contrary, PMS exhibited better metal chelating than PGS when the concentration of the polymer was higher than 0.2 mg/ml. The antioxidant abilities of sulphated alginate derivatives can be considered as an important factor for potential drug delivery application.

Keywords: Alginate; Sulfated polymannurnic acid (PMS); Sulfated polyguluronic acid (PGS); Antioxidant activity

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P14-3

Development of a promising electrospun bacterial cellulose-PHB scaffold for tissue engineering

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In recent years, biopolymers have become frequently used biomaterials in various fields due to their biocompatibility and biodegradability. Depending on the backbone structure, several types of biopolymer are produced by a different type of microorgan-isms. Bacterial cellulose is one of them produced by *Gluconobacter*, *Agrobacterium* and *Achromobacter* genus. Although the structure of bacterial cellulose is similar to that of plant cellulose, its chemical and physical properties are so different. Due to its water absorp-tion capacity, high crystallinity grade and high strength properties, bacterial cellulose has become a preferred biopolymer in various areas such as food, paper, and health industry. Another impor-tant biopolymer synthesized by microorganisms such as *Ralstonia eutropha* and *Bacillus megaterium* is polyhydroxybutyrate (PHB). Like bacterial cellulose, PHB, a member of the polyhydroxyalka-noates, is a promising biopolymer because of its biocompatible and environmentally friendly properties. Recently, co-usage of different polymers to consolidate their properties has become widespread.

One of the most substantial applications of polymer blends is the production of tissue scaffolds. For that reason, in the present work, it was aimed to produce cellulose from *Komagataeibacter hansenii* DSM 5602 and PHB from an obligate alkaliphilic, gram-positive bacterium *Bacillus marmarensis* GMBE 72^T. In conclusion, these polymers were then blended and a scaffold for tissue engineering was produced by using electrospinning technique.

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P14-4

Optimal ring-opening polymerization for producing surface-modified cellulose nanofibers-graft-poly(lactic acid)s

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Surface-modified cellulose nanofibers were prepared from softwood cellulose fibers (white spruce bleached kraft pulp) by 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation in an aqueous TEMPO/NaBr/NaClO system and subsequent postoxidation with $NaClO_2$ in acetate buffer (pH = 4.8). The resulting TEMPO-oxidized cellulose nanofibers (TOCNs) exhibited a high crystallinity of 67–69% with a high density of carboxylate content of 1.7 mmol g^{-1}. These TOCNs were used to produce highly-crystalline TOCN-*graft*-poly(lactic acid) (PLA) nanocomposites (TOCN-*g*-PLA) *via* ring-opening polymerization (ROP) of L-lactide in dimethyl sulfoxide (DMSO). The reaction temperature (80, 90, 100 °C) and molar ratio of L-lactide to carboxylate surface groups (R = 1:1, 5:1, 10:1) were optimized to potentially enhance the formation of covalent links (i.e. ester formation) between the PLA and the TOCNs to form a strong nanocomposite. The TOCN-*g*-PLA nanocomposite with the highest production rate of the ester bond (Q_P = 0.028 h^{-1}) and the highest yield of the ester formed on carboxylate content ($Y_{P/S}$ = 0.624), was successfully produced in DMSO at a temperature of 90 °C and R = 10:1. Solvent dispersibility of the TOCN-*g*-PLA samples in DMSO (a polar aprotic solvent) and chloroform (nonpolar organic solvent) was improved relative to natural cellulose nanofibrils. The process used in producing the nanocomposite enhanced covalent bonding between the modified cellulose nanofibrils and the polymer matrix, to improve the mechanical and thermal properties of the product.

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P14-5

Nanofibres from bacterial cellulose as a composite material

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In recent years there has been still growing interest in cellulose nanostructures such as nanofibres, nanofibrils and nanowhiskers. Some bacterial genera e.g. *Gluconacetobacter*, *Sarcina* and *Agrobacterium* secret extracellularly cellulose as a nanofibre network. Bacterial cellulose (BC), sometimes called nano-bio-cellulose, in contrast to the plant cellulose is devoid of hemicelluloses, lignins and pectins and possesses unique physical and mechanical properties resulting from its three-dimensional nano-sized fibrous structure. Based on BC a range of medical products has been developed including dressings for treating burns and trophic wounds, bone implants, temporary skin, artificial blood vessels and scaffolds for tissue engineering. Studies on BC, however, are conducted also in other fields such as composite materials. Although mechanical properties of bacterial cellulose do not exceed those of petroleum-based polymers, nano-fibrous composite materials based on BC and synthetic polymers can be suitable for various technical applications, especially for structural materials. Among the factors limiting the range of potential applications of BC is a relatively high cost of the nutrient medium required for the cultivation of bacteria. Development of alternative low-cost culture media for BC-producing bacterial strains would increase the competitiveness of this unique material, thereby increasing the range of economically justified practical applications including the field of structural, composite materials.

In present work, the waste from the food industry, as well as liquid from enzymatic maceration of fibrous plants, was used as an ingredient to BC biosynthesis medium. Nanofibres obtained by disintegration of the BC films were used as components of biodegradable composites.

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P14-6

Synthesis of polyvalerolactone via enzymatic ring opening polymerization in different reaction media

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The nature of employed solvents plays a crucial role in determining the stability of the biocatalyst and in the partitioning of substrates and products between the solvent and the biocatalyst in non-aqueous biocatalytic systems. In the current study, δ-valerolactone ring opening polymerization was carried out in different reaction media such as hexane, toluene and without solvent with both commercial enzyme, Novozyme 435 and with Candida antarctica lipase B (CALB) immobilized onto rise hush ash (RHA) via physical adsorption. For the determination of optimum reaction medium for enzymatic poly(δ-valerolactone) (PVL) synthesis, polymerizations were performed at various reaction periods (6, 24, 48, 72, and 120 h) and reaction temperatures (30, 40, 60, and 80 °C) for each reaction media. Molecular weight distributions and chain structures of the polymer samples were compared by gel permeation chromatography (GPC). The highest molecular weight (Mn = 9200 g/mol) was obtained at 40 °C at the end of 24 h in hexane medium via Novozyme 435. On the other hand, optimum reaction medium was toluene for the immobilized CALB. The highest molecular weight was reached as 8020 g/mol at 80 °C after 120 h.

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P14-7

All about blocks . . .and copolymers thereof – current and future playgrounds for polyhydroxyalkanoates

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Our work aims at the enzymatically catalyzed modification of biorenewable and biodegradable polyhydroxyalkanoates (PHA) to form well-defined block-copolyesters. Such materials are recognized to expand the range of PHA properties and molecular architectures (i.e. viscosity, flexibility, rigidity, crystallinity, block-copolymer systems, crosslinkable systems) and have gained special attention due to their potential for high value added applications (e.g. resorbable implants, tissue engineering, drug delivery and smart materials). While current chemical synthesis strategies encumber economically realistic access due to reaction complexity, a distinct need for new methods of their preparation has been identified.

Two methods with a different range of combinations of PHA block segments are under investigation. The first method employs genetically engineered bacteria and diligent substrate feed techniques to synthesize in vivo chiral, block-copolymeric PHAs.

The second method utilizes the provided polymeric material from fermentative biotransformation as substrates for their enzymatically catalyzed conversion into novel block-copolymeric PHAs and thus eliminating the use of bio-incompatible reagents, reactants or catalysts.

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P14-8

Improvement of BC composites properties for dressings material

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Cellulose is the most abundant biopolymer in the world and normally is extracted from plants, where it occurs in the presence of hemicellulose and lignin. Bacterial cellulose (BC), synthesized by *Komagataeibacter xylinus*, is an extremely pure form of cellulose, not contaminated with other compounds. The scientific and commercial interest in BC, especially in biomedical applications results from its properties such as biocompatibility, high mechanical strength and water holding capacity [1].

The wound dressing made from BC maintain the proper moisture level and constant temperature of the wound bed which can significantly accelerate wound healing process with renewal of skin cells. BC modified with different polysaccharides have changed properties, which extend the possibilities of using BC in medical application [2,3].

The study describes the preparation and characterization of bacterial cellulose composites, which can be used as a wound dressing material for treatment of chronic wound. Addition of CMC to culture medium, which interfered with the formation of BC structure [2], results in denser structure with thinner fibres and reduced crystallinity [3]. That architecture is preferable for the highest rehydration ratio and can be also intensified by glycerol addition. Furthermore, glycerol is used as biocompatible plasticizer, which strongly interacts with cellulose fibers by H-bond and increases its tensile strength. Also the fibroblasts viability, tested in vitro, were slightly higher in comparison to pure BC and control on plastic.

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P14-9

Extraction, purification and modification of poly (3-hydroxybutyrate) produced by the fermentation of fatty acids with *Burkholderia cepacia* B27

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Poly (3-hydroxybutyrate) is a thermoplastic polyester of the family of the polyhydroxyalkanoates produced by different microorganisms under stress conditions. It has very interesting mechanical and physicochemical characteristics that allow it to be used for packaging applications. However, it's necessary to improve some of its weakest characteristics like the high brittleness, the production cost and the narrow processing window. This improvement can be achieved using different extraction, purification and modification technics that result in materials with different physicochemical characteristics and production costs.

In this work different extraction, purification and modification processes were evaluated in order to obtain a polymer with competitive mechanical and thermal properties. Fatty acids were used as carbon source and the fermentations were made in 5L, 20L and 100L bioreactors. Initially, the polymer was extracted and separated from the surrounding PHA hyper-productive mutant bacteria *Burkholderia cepacia* B27 biomass, using techniques like chemical digestion with SDS and NaOH, centrifugation and solvent precipitation. Then the polymer was purified to remove residues from the fermentation, for this, the performance of different non halogenated solvents was tested under different conditions. Finally, the polymer was modified blending it with other biodegradable polymers like polyethilenglycol and adding different organic fillers to evaluate improvements in the mechanical characteristics. The samples were characterized by TGA and DSC essays and different mechanical tests. The results indicate that the use of organic polar solvents such as methanol and ethanol allow to obtain a colorless, odorless and high purity polymer. Also, the use non halogenated solvents avoid the use of chloroform in the process, which is a hazardous solvent that can't be used at the industrial production level.

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P14-10

Withdrawn

P14-11

Lipase-catalyzed synthesis and characterization of poly(Ω-pentadecalactone): an alternative aliphatic polyester to poly(ε-caprolactone)

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There is an increasing interest on enzymatic ring opening polymerization (eROP) of unsubstituted lactones since the end products are acceptable alternatives for petroleum based polymers with convenient physical properties and biodegradability. There exist limited studies on eROP of macrolactones, such as 16-memberedω -pentadecalactone (ω -PDL). Moreover, poly(ω -pentadecalactone)(PPDL) is comparable with low density polyethylene (LDPE), since it has similar thermal and mechanical properties. When compared to poly(ω -caprolactone) (PCL), PPDL has higher melting point (60 °C and 97 °C, respectively) which provides tolerance to be used in higher temperatures. In literature, generally Novozyme 435 (the commercial immobilized form of *Candida antarctica* lipase B) has been preferred for eROP of macrolactones. In this study, a new biocatalyst *C. antarctica* lipase B (CALB) immobilized onto rice husk ashes (RHA) via physical adsorption (Im-CALB) was proposed. The successful performance of this enzyme for PCL synthesis was shown in previous study. Polymerization reactions were carried out in 1 g of toluene under inert nitrogen atmosphere at various temperatures (60 °C, 80 °C, and 90 °C) and reaction periods (0.5–24 h). Monomer conversions and molecular weights (M_n) were calculated from Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra. Chemical structure of the polymers was examined via both ^{1}H NMR and Fourier Transform Infrared Spectroscopy (FTIR). For the thermal characterization of the polymers, Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) were applied. Highest molecular weight (M_n = 34200 g/mol) was reached at 80 °C at the end of 6 h. Consequently, successful enzymatic synthesis of PPDL was performed and it was suggested for biomedical applications as an alternative to PCL.

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P15-1

Effect lipids in palm oil mill effluent on process imbalance of biogas production systems

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Lipids are one of the major organic pollutants in palm oil mill effluent (POME). The anaerobic digestion (AD) process is often inhibited by lipids and long chain fatty acids (LCFAs). This study aimed to elucidate effects of lipids and long chain fatty acids (LCFAs) in POME on methane production and changing of microbial communities. The initial organic loading of 10 g-VS/L corresponding to lipids concentration of 1.85 g/L show inhibition effect in biochemical methane potential test of raw POME. In batch test, POME added LCFAs and glycerol was resulted to decreasing methane yield. POME overloaded was number of hydrolytic and acetogenic bacteria increased and cause to a shift hydrogenotrophic archaea. In the long term, the process could self-recovered after inhibition by LCFAs except glycerol appeared accumulated of VFA and directly result to failure process. The metagenomic sequencing showed POME overloaded increasing number of genera Rikenellaceae, Planococacea and Methanosaeta.

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P15-2

Expression and thermal stability of an 11S globulin modified in both, third and fourth variable region

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The functionality of acidic subunit from amaranth 11S globulin has been improved in this work by inserting antihypertensive peptides. Two modified proteins, one modified into third variable region (ACC3) inserting four VY peptides in tandem; and other one, doubly modified in both third and fourth variable region

(ACC3.4) with insertion of four VY in tandem in the third variable region and IPP peptide in fourth variable region. Both ACC3 and ACC3.4 antihypertensive proteins were expressed separately in *E. coli* BL21 codonplus (DE3)-RIL obtaining 0.44 and 0.33 g/L of recombinant protein respectively. The recombinant proteins were extracted from inclusion bodies. After purification and refolding, the proteins were subjected to thermal denaturalization by circular dichroism, showing that thermal stability was affected with respect to the protein unmodified since curve transition were not observed in the modified proteins, which suggests that possibly the both proteins are taking a structure as molten globule form. These mutant proteins have seven times more antihypertensive activity than the unmodified protein, and the structure may have some applications as functional food ingredients.

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P15-3

Phase behavior and physical properties of elastin-like polypeptide (ELP) block co-polypeptides: effects of drug delivery carriers

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The development of environmentally responsive drug carriers requires a new method for assembling stimuli-responsive hydrogel. Recent progress suggests that short peptide motifs can be engineered into biopolymers with specific temperature-dependent behavior. Among them, thermo-reversible micelles derived from ELP have a significant advantage in that they can be synthesized with near-absolute control of macromolecular architecture using genetic engineering techniques.

In this study, ELP block co-polypeptides containing hydrophobic and hydrophilic segments were synthesized and examined as a drug delivery carrier by formation of micelles. Physical properties of these micelles were observed by lower critical solution temperature (LCST), dynamic light scattering (DLS), and small angle X-ray scattering (SAXS) analyses. In addition nano-encapsulation and release capacity of a model drug were investigated. Through these experiments, it was confirmed that ELP block copolymers stably formed thermo-responsive micelles, and that they were good candidates as temperature-sensitive drug delivery carriers.

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P15-4

Rational generation of anti-fibroblast growth factor receptor 2 antibody for gastric cancer therapy

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Fibroblast growth factors (FGFs) and their receptors control a wide range of biological functions. Dysregulated FGF signaling has been implicated in the pathogenesis of human cancers. Aberrant activation of fibroblast growth factor receptor 2 (FGFR2) signaling, through overexpression of FGFR2 has been found in a variety of human tumors such as gastric cancer. For target therapy, antibodies may have substantial benefits by relying on their specificity to FGFR2. Cancer patients with aberrantly activated/amplified FGFR2 signaling could potentially benefit from therapeutic intervention with FGFR2-targeting antibodies. In this study, we have generated anti-FGFR2 single-chain variable fragment (scFv) from immunized chickens by using phage display technology. Based on the findings, one isolated scFv F2pS3 can block the active site of FGFR2 to prevent FGFs access and has the ability to inhibit cell growth of gastric cancer. ScFv F2pS3 is able to recognize endogenous FGFR2 on the cancer cells and inhibit downstream cell growth signals. Moreover, scFv F2pS3 also exhibits excellent effect of angiogenesis inhibition was found in the animal model. Further, to study the inhibition effect of the humanized IgG F2pS3 against tumor growth in vivo, the NOD-SCID mice were inoculated with the SUN16 cells and intravenous injection of humanized IgG F2pS3 once weekly inhibited the growth of SNU16 xenograft. The complex structure of humanized scFv interacting with FGFR2 also have been constructed by molecular modeling to clarify antibody to interfere with ligand-receptor interaction. We believe that humanized IgG F2pS3 is potential to be developed for cancer therapy in the future.

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P16-1

Resolving the complexity of ACS7-PP2C protein complex formation and its impact on ethylene biosynthesis

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Plant hormones abscisic acid (ABA) and ethylene (ET) play crucial role in plant adaptation to abiotic stress. The key enzyme in ethylene biosynthesis is ACC synthase (ACS, 1-aminocyclopropane-1-carboxylate synthase) that catalyzes the conversion of S-adenosyl methionine (SAM) to 1-aminocyclopropane 1-carboxylate acid (ACC). The Arabidopsis genome contains 12 genes annotated as ACS. Based on the C-terminal sequences, ACS proteins were divided into three main types (I-III). Type III ACS is represented by a single protein – ACS7 that has longer N-terminal region and C-terminal fragment lacking the MAPK and CDPK phosphorylation sites. The unique structure of ACS7 contribute to its function and regulation which are far from understanding. It is know that group A PP2Cs (ABI1/HAB1/ABI2/AHG1) which are known as negative regulators of ABA signaling significantly alter ACS7 protein function or fate. Here we present an insight into mechanism of interaction between ACS7 and PP2C group A phosphatases. To better understand ACS7-ABI1PP2C protein complex formation ACS7 homodimer model was generated and assembled with experimentally determined PP2C protein structures. Based on this analysis potential ACS7-PP2C binding sites were proposed and macromolecular docking was performed. Furthermore, to test the validity and accuracy of bioinformatics predictions bimolecular fluorescence complementation (BiFC) and fluorescence resonance energy transfer (FRET) analyses were carried using both the wild type and mutant ACS7/PP2C proteins. Overall, this study using combinatorial approaches has contributed to understanding of the mechanism by which ABA regulate ethylene biosynthesis.

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P16-2

Systemic analysis of multiple coordinated changes associated with clavulanic acid overproduction in an industrial strain of *S. clavuligerus*

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The classical strain improvement methodology relying on iterative cycles of mutagenesis followed by screening is still fascinating in the post-"omics" era for its long-standing history and successful application [1]. This approach finds a notable application in the field of secondary metabolite production. *S. clavuligerus* DEPA strain used for clavulanic acid (CA) manufacturing process in Turkey for many years produces at least 100-fold relative to the standard strain *S. clavuligerus* NRRL3585. We recently reported a comparative analysis of cytosolic proteome of these two strains as based on 2DE followed by protein identification via MALDI-TOF/MS [2]. The differentially-expressed proteins corresponded to 33 distinct ORFs for underrepresented ones and 60 ORFs for overrepresented ones, and mainly belonged to the functional classes namely "general function", "amino acid metabolism", "hypothetical/unknown", and "secondary metabolism". A more comprehensive understanding of multiple coordinated alterations of CA overproduction were next obtained by broadening the range of proteins covered by taking LC-MS/MS approach as well and parallel quantitative transcript analyses. LC-MS/MS could identify ca. 160 differentially expressed proteins, excluding those that were already identified by gel-based approach. Overrepresented ones were mostly related with the regulation of transcription and translation while those underrepresented were mostly the proteins of the central metabolism of the organism (amino acid and lipid metabolism, in particular). Q-RT PCR of the selected genes revealed that the protein abundance is primarily determined by the transcript levels. Taken together, our systemic analysis provided a rather useful insight into the genotype-phenotype landscape of the overproducer strain [3].

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P16-3

Proteome-wide analysis of pleiotropic effects of targeted homoserine dehydrogenase (hom) gene disruption in *Streptomyces clavuligerus*

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In our earlier study, we undertook targeted *hom* disruption in *Streptomyces clavuligerus* to improve cephamycin C (CC) yields. In this way, all the carbon coming from aspartate would be directed to L-lysine rather than being shared between the two branches, and secondly, concerted feedback inhibition of aspartokinase would be relieved. This rationale manipulation resulted in up to 4.3-fold and 2-fold increase in intracellular free L-lysine concentration and specific cephamycin C (CC) production, respectively, during stationary phase in chemically defined medium supplemented with L-methionine and L-threonine [1]. There appeared some other consequences of this mutation on cellular physiology since the mutant displayed some developmental alterations as well as red pigmentation. The present study thus focused on global determination of the impact of *hom* inactivation on primary and secondary metabolic pathway fluxes as well as other physiological functions of our mutant. For this aim, comparative proteomics via LC–MS/MS was taken as an approach followed by quantitative transcript analysis of the selected genes. The study identified 68 differentially expressed proteins, 28 being overrepresented and 40 underrepresented. Apart from many hypothetical/unknown proteins, the identified proteins mainly belonged to the functional classes namely secondary metabolism, amino acid and lipid metabolism, and DNA replication, repair and transcription [2]. Subsequent Q PCR results clearly indicated that protein levels accord well with the transcript levels for the majority of the genes analyzed.

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P17-1

Targeting the adiponectin expression in ADSCs by a small molecule and its beneficial therapeutic effect on the type 2 diabetes mouse model

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Adiponectin is an anti-diabetic adipokine secreted by adipocytes and has been found related to metabolic dysfunctions, inflammation and insulin resistance. Adipose-derived stem cells (ADSCs) show great clinical potential to treat type 2 diabetes due to their abilities for regulation/secretion of adipose-associated cytokines. We found that the expression level of adiponectin in ADSCs could be incAreased by a small molecule TW03. The aim of this study was to evaluate the therapeutic effect of TW03-pretreated ADSCs' transplantation using high fat diet (HFD)-induced type 2 diabetic mouse model and to investigate the mechanism for the regulation

of adiponectin expression in ADSCs by TW03. In comparison with the untreated ADSCs, it was found that TW03-pretreated ADSCs led to the improvement in key parameters related to obesity and insulin resistance, including fasting blood glucose, insulin, triglyceride, total cholesterol levels, HbA1c, IL-6, IPGTT and HOMA-IR values. The therapeutic benefits may be due to the high amount of adiponectin from ADSCs activated paracrine AKT and AMPK signaling pathways in hepatocytes. Because obesity (BMI, waist girth and LDL-cholesterol level) are associated with DNA hypermethylation of adiponectin gene, the up-regulation of adiponectin expression by TW03 treatment may be through an epigenetic control. To test whether TW03 could reduce DNA methylation statuses in adiponectin CpG islands, bisulfite sequencing was used to examine the DNA methylation status. The mRNA levels of key adiponectin regulatory transcription factors, including *PPARγ2* and *C/EBPα*, were not significantly altered in TW03-pretred ADSCs. This study provides putative therapeutic mechanisms and a novel strategy including a small molecule treatment combined with ADSC transplantation that delivers the therapeutic benefits of type 2 diabetes.

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P18-1

Functionalized filter paper-based 3D liver model for *in vitro* drug screening applications

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The current market in the healthcare and diagnostic sector demands the development of a cheap, affordable and portable 3D liver model for high-throughput drug screening. An efficient liver model would provide a much required in vivo mimicking microenvironment to the cells and would support their long-term viability and functionality. With this perspective, this study delineates the fabrication and in-depth characterization of filter paper-based 3D liver model. We functionalized the filter paper with (3-Aminopropyl)triethoxysilane (APTES) to enhance its cell adhesion capacity. To further improve its biological response, the paper was coated with the caprine liver-derived extracellular matrix. The fabricated substrates supported significantly higher adhesion, proliferation, and viability of the human liver cells as compared to the non-functionalized paper matrix. The cells exhibited significantly higher metabolic functionality i.e., high albumin secretion, urea production, and high expression of mature hepatocyte markers. We have also demonstrated the application of the paper-based 3D liver model for acetaminophen-induced toxicity evaluation. The results showed a significantly higher sensitivity of the hepatocytes in paper-based 3D culture as compared to 2D monolayer culture, similar to that of physiological drug-induced responses. In conclusion, APTES functionalized filter paper could serve as an efficient biomaterial platform for the development of microscale technologies, liver-associated diagnostics, and drug screening applications.

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P18-2

Filter paper-based 3D liver model *in vitro* drug screening applications

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The current market in the healthcare and diagnostic sector demands the development of a cheap and portable 3D liver model for high-throughput drug screening. An efficient liver model would provide the cultured cells, much required in vivo like microenvironment and would support their long-term viability and functionality. With this perspective in mind, this study delineates the fabrication and in-depth characterization of filter paper-based 3D liver model. The absence of any cell adhesion motif limits its application for cell culture; therefore, we functionalized paper with (3-Aminopropyl)triethoxysilane (APTES). To further improve its biological response, the paper was coated with liver-derived extracellular. The fabricated substrates supported significantly higher adhesion, proliferation, and viability of the human liver cells as compared to the unfunctionalized paper matrix. Moreover, the cells exhibited significantly higher metabolic functionality i.e., high albumin and urea section, high expression of mature hepatocyte markers, and high rate of acetaminophen detoxification. Furthermore, we showed the application of the paper-based 3D liver model for drug toxicity evaluations. The results showed a significantly higher sensitivity of the hepatocytes in paper-based 3D culture as compared to 2D monolayer culture, similar to that of physiological drug-induced responses. In conclusion, APTES functionalized filter paper could serve as an efficient biomaterial platform for the development of microscale technologies, liver-associated diagnostics, and drug screening applications.

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P18-3

Effects of progesterone on in vitro culture of murine endometrium

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Uterus, the major organ for reproduction, is one of the most elastic organs in human body. They consist with several types of cells including myometrium and endometrium. Endometrium is an outer layer of uterus tissue and responsible for implantation of embryo. Therefore, the regeneration of endometrium is major issue in field of infertility. In this study, we tried to establish proper condition for proliferation using progesterone and analyzed the expression of miRNAs.

Murine uterus horns were isolated from 6-week-old C57BL/6 mice, dissociated using collagenase type I and replated. Human endometrial carcinoma cells, SNU-1077, was purchased from Korean Cell Line Bank. Immortalized human endometrium cell line, CRL-4003, was purchased from ATCC and the cells were treated with different concentrations of progesterone. The expression of miRNA was analyzed using qRT-PCR.

The expression of miR-15b, -29a, -197, and -200c was altered according to progesterone concentration in different endometrial cell lines. Additionally, expressions of VEGF-A, MUC1, TIMP-1, MMP-2 were up-regulated due to the progesterone treatment.

In conclusion, we demonstrated the optimal in vitro culture condition of endometrium and the progesterone could be a major modulator for expression of early pregnancy genes (2016R1E1A1A01943455).

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P18-4

Methylation analysis of transgenes in transgenic pigs and rabbits

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Epigenetics involves studies of changes in gene expression not associated with changes at DNA sequence level. The best known epigenetic mechanism involved in control of gene expression is DNA methylation. Methylation of cytosines in CpG island within promoter region usually causes gene silencing. In transgenic animals we are interested not only in introduction of transgene but also in its activity.

We have prepared transgenic pigs for xenotransplantation purposes with decreased recognition by human immune system by introduction of the human HLA-E gene under elongation factor 1 alpha promoter (EF-1α) and transgenic rabbits expressing human growth hormone hGH under WAP promoter in mammary gland of lactating females. Activity of both transgenes was already confirmed on molecular level and using flow cytometry (HLA-E) or radioimmunoassay (hGH). The purpose of this study was to evaluate specific methylation of both transgenes. Genomic DNA was modified by bisulfite conversion and PCR products were analyzed using pyrosequencing method. Pyrosequencing enabled quantification of the specific methylation level of transgene. The total level of methylation of the HLA-E transgene promoter was approximately 90% (range 67–100%) and the HLA-E gene 73.79% (29–100%). In the case of hGH transgene total level of methylation was about 75.2% (range 55.7–98.5%). This suggest that level of methylation of transgene does not affect directly its biological activity.

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P18-5

OncoCilAir™: A physiological *in vitro* platform to assess the efficacy and the toxicity of lung cancer therapeutics

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Every year, lung cancer is the most frequently diagnosed cancer in men and women leading to 1 million deaths worldwide. Clearly, realistic human 3D models, which recapitulate heterologous interactions between epithelial, stromal and tumor cell types are required to improve preclinical predictivity. In this prospect we have developed OncoCilAir™, a Non Small Cell Lung Cancer in vitro microtissue model which combines a functional reconstituted human airway epithelium, human primary lung fibroblasts and lung adenocarcinoma cell lines cultured at the air-liquid-interface. Remarkably, in this 3D microenvironment tumor cells expand by forming nodules, closely mimicking human lung cancer features. In addition, multiphoton imaging experiments revealed that OncoCilAir™ replicates in vitro the ability of tumors to influence surrounding healthy tissues, underscoring the physiological relevancy of the model. Accordingly, drug screenings results demonstrated that OncoCilAir™ allows accurate ranking of drug candidates through the simultaneous assessment of their efficacy and side-toxicity. Moreover, since the model remains functional for several months and reproduces in vitro critical pitfalls of the lung airway like mucus secretion and cilia beating, we showed that it can be used to assess functionalised nanoparticles, oncolytic viruses but also inhalation therapies through controlled nebulisation. Lastly, we used genome editing tools to demonstrate the feasibility of genetically modifying in vitro engineered human microtissues. In doing so, we successfully induced carcinogenesis in smoker microtissues. In conclusion, OncoCilAir™ heralds a new generation of in vitro genetically editable human models which is expected to accelerate the development of optimal lung cancer therapies while reducing animal testing.

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P19-1

Engineering the methylerythritol phosphate pathway in cyanobacteria for photosynthetic isoprene production from CO_2

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Terpenoids are used in diverse markets as pharmaceuticals, nutraceuticals, cosmetics, and disinfectants. The simplest terpene, isoprene, is a key building block of synthetic rubber and currently produced entirely from petrochemical sources. To produce isoprene directly from CO_2, we engineered the isoprene biosynthetic pathway in the cyanobacterium *Synechococcus elongatus*, with guidance provided by dynamic flux analysis and metabolite profiling. The methylerythritol phosphate (MEP) pathway was selected for cyanobacterial isoprene synthesis based on comparison of carbon efficiency and precursor driving force between MEP pathway and mevalonate (MVA) pathway. We identified the pathway bottlenecks and increased the isoprene biosynthetic flux by overexpressing bottleneck enzymes, optimizing precursor levels, and constructing enzyme fusions. The engineered strain directed about 40% of photosynthetically fixed carbon toward the isoprene biosynthetic pathway, resulting in the production of 1.26 g L^{-1} of isoprene from CO_2, which is a significant increase for terpenoid production by photoautotrophic microorganisms. The strains developed in this study can be used to construct a photoautotrophic cell factory for the production of diverse terpenoids from CO_2. In the second part, I will introduce our work on identification of the synergy between the MEP pathway and the MVA pathway for isoprene production in *Escherichia coli*. We observed that simultaneous utilization of MEP pathway and MVA pathway resulted in significant increases in the fluxes through both pathways. Our results strongly suggest that coupling of the complementary reducing equivalent demand and ATP requirement plays an important role in the synergy of the dual

pathway. The synergy of MEP pathway and MVA pathway can be used to improve the production of a broad range of terpenoids in microorganisms.

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P19-2

Enzyme scaffolding for metabolic engineering endeavors

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Production of valuable metabolites at high titers is often hindered by interference with other metabolic processes or toxicity of pathway intermediates. Spatial organization of enzymes has the potential to overcome these problems and facilitate metabolic engineering. This can be achieved for instance by docking enzymes onto co-expressed synthetic scaffolds.

Here we suggest scaffolds made out of defined RNA modules. One RNA module contains two or more stem-loop structures called aptamers that function as docking domains for proteins through specific peptide-RNA interactions. Proteins of interest, for instance enzymes of a metabolic pathway, can be targeted onto the scaffold simply by fusing peptides that can bind to the aptamers. Targeting efficiency is evaluated in several *Escherichia coli* strain backgrounds by using a bimolecular fluorescence complementation (BiFC) assay measured with flow-cytometry. Co-expression of tagged split-YFP variants together with a corresponding RNA-scaffold brings the split parts in close proximity upon scaffold docking and promotes refolding into functional YFP. This results in an increase in signal intensity compared to protein expression without scaffold.

Applying the designs for chemical production using an engineered *E. coli* MG1655 strain, enzymes of the itaconic acid pathway are targeted onto the RNA-scaffold. Enzyme binding to RNA-scaffold is also confirmed in vitro using a band-shift assay (EMSA). Co-expression of tagged enzymes and RNA-scaffold show an increased specific productivity within the first 48 h of batch fermentation compared to strains without scaffold expression.

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P19-3

Fine-tunable knockdown system based on modulation of synthetic sRNA expression levels in *Escherichia coli*

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Fine-tuning gene expression is essential for optimization of genetic networks, but conventional methods are time-consuming and laborious. Here, we developed a fine-tunable knockdown system based on varying small RNA (sRNA) expression levels. We observed the proportional relationship between sRNA expression levels and target gene repression by using two different genes, DsRed2 and pgi. As proof-of concept examples, we applied synthetic sRNAs libraries with various expression levels, achieved using different-strength promoters, to putrescine- and L-proline-producing strains, and isolated *E. coli* strains with high-production capability. Weak co-repression of argF and glnA led to the highest putrescine titer of 43.0 g/L, a value that is 77.7% higher than that obtained from the previously reported best strain, XQ52 p15SpeC (24.2 g/L). In contrast, strong repression of the same target genes resulted in the L-proline titer of 32.7 g/L from the additional engineered strain sharing a precursor (L-ornithine) and metabolic pathway with putrescine producing strain. Fine-tuning gene expression by controlling sRNA expression levels will be a useful metabolic network-optimization strategy because it is a fast, simple, and cost-effective process that requires no sRNA modifications [1]. [This work was also supported by the National Research Foundation of Korea grant funded by the Ministry of Education, Science and Technology (2017R1A2B4004447).]

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P19-4

The dimerization buffers noise at protein level

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The gene expression is subject to random fluctuations or noise. The noise of regulatory protein is usually considered detrimental in the context of regulation. The discovery of a new strategy to buffer noise at the protein level is important in synthetic biology. In this study, we proposed a new noise-buffering motif applicable to most of the regulatory networks. We demonstrated that the reaction of dimerization has potential to reduce the noise of regulatory proteins. This novel design is capable of attenuating both external and internal noise. With stochastic simulation algorithm (SSA), we found that the dimerization buffered more noise than that of negative feedback control or incoherent feedforward loop. Specifically, the coefficient of variation (COV) of a regulatory protein in case of dimerization is less than half of that in the case of negative feedback control or incoherent feedforward loop. Moreover, the dimerization buffered noise at the stage after translation, which grants the attenuation of the translation noise in time.

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P19-5

Phospholipid vesicles to determine the transport functionality of mitochondrial carrier proteins

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Mitochondrial carrier proteins play a key role in many metabolic pathways. Some of these membrane transport proteins carry di- and tricarboxylic acids across the mitochondrial membrane. The construction of efficient cell factories to produce secondary metabolites requires a deeper understanding of transport capabilities. However, dissecting these highly hydrophobic proteins requires the development of suitable expression systems and membrane-like environments to retain their functionality.

In our study, we apply synthetic phospholipid vesicles to incorporate fully functional membrane proteins for transport activity assays. In the formed proteoliposomes, membrane transporters are

embedded into a phospholipid bilayer, which surrounds an aqueous inner compartment. These constructs enable the simulation of distinct cellular compartments in vitro due to possible variations in the composition of phospholipids, integral membrane proteins and enclosed inner substances. We established a cell-based and a cell-free process to construct these systems, ranging from the expression to the incorporation of membrane transporters into vesicles. Usage of proteoliposomes in combination with specific enzymatic assays allows the analysis of the carrier process. The information obtained helps to develop metabolic models and metabolic engineering strategies incorporating details about transport processes, which has great potential to push the frontiers of microbial organic acid production.

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P20-1

Systems-biology based synergistic multidrug combinations that modulate mitosis and induce a senescence-associated phenotype

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Cancer remains the global health problem and new treatments are needed to improve compliance, combat resistance and secure better safety profile. Ideally such treatment would be composed of multiple drugs with synergistic interactions. However, such drug optimization remains an open challenge due to the immense number of possible drug-dose possibilities to be experimentally tested.

We used a phenotypic systems-biology method, based on a simple *in vitro* assay of cell viability and data modeling, that rapidly identified an optimal multidrug combination (ODC). This ODC was specific to cancer cells and inactive in non-cancerous cells. Moreover, the ODC-induced formation of chromosome bridges that persisted after mitotic exit and delayed abscission in cancer cells, therefore suggesting initiation of cellular senescence.

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P20-2

Integrative genome-wide analysis of long-term effects of doxorubicin on yeast cells

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Doxorubicin is a chemotherapy agent commonly used in treating different cancer forms. The mechanism(s) of the action of doxorubicin has not been resolved due to the contrasting findings. In order to elucidate the long-term cellular effects of doxorubicin, an integrative systems biology approach was followed in *Saccharomyces cerevisiae* cells. The investigation of genome-wide transcriptomics and genome-wide fluxes in the presence of doxorubicin, two independent quantifications, consistently showed similar results on the effect of doxorubicin: the up-regulation of genes involved in response to oxidative stress as well as in Rad53 checkpoint sensing and signaling pathway. Mainly, glycolysis, amino acid biosynthesis, one carbon metabolism and sulfate assimilation processes were repressed. Further, transcriptome and interactome data were integrated to identify active networks. Modular topological analysis indicated the induction of the genes significantly associated with nucleosome assembly/disassembly and DNA repair in response to doxorubicin. Transcription factors represented in each module were identified to determine the likely co-regulation. All these observations indicate not only that doxorubicin generates reactive oxygen species and DNA damage, but also it induces an impressive re-wiring of metabolic and signalling pathways, which are suggested to be therapeutic targets in cancer.

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P20-3

Systems biology approach to elucidate life cycle of yeast dsRNA virus

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Double-stranded RNA (dsRNA) viruses of Totiviridae family from the industrially relevant yeast *Saccharomyces cerevisiae* are widely spread among fungi and identified in insects and plants, also. This group includes ubiquitous yet poorly understood benign inhabitants of the yeast, unable to alleviate cell growth or confer other detectable phenotypic changes. We focus on systems biology approaches to address the molecular mechanisms of dsRNA viruses use to survive in the host cell. The developed techniques allowed performing genomic, transcriptomic and proteomic analyses to address the establishment of Totivirus in host cell and extracellular relations of yeast bearing such viruses.

Basing on developed virus genome cloning technique, virus genes were re-introduced into host. Over-expression of modified L-A capsid protein resulted in complete clearing of genuine virus in a robust and controllable fashion. Comparative transcriptomic studies deciphered distinct pathways involved in maintenance of different dsRNA viruses. Conversely, boosted synthesis of viral genome was achieved by over-expression of L-A Gag-Pol protein, making viral dsRNA the most prevalent form of individual RNA molecule in a cell without observable impact on cell growth parameters. Taking advantage of controlled expression of virus-like particles (VLPs) with or without viral genome, we purified them and addressed host factors, associated with various virus conditions in a cell. Proteomic analysis of VLPs identified host proteins specific for certain phenotypes, thus characterising host factors involved in different steps of life cycle of a L-A virus. This research was funded by Research Council of Lithuania, grant #SIT-07/2015.

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P21-1

Dissection of growth-rate- and cell-cycle-dependent gene regulation in *P. pastoris*

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Protein secretion in *P. pastoris* is coupled to the specific growth rate and it was established that at least in case of constitutive expression of the recombinant gene, the specific secretion rate is positively correlated to growth. Furthermore, it has been demonstrated that budding yeast is more secretion competent in the G_2/M phase of the mitotic cell cycle. As in budding yeast growth and cell cycle are tightly interconnected, this raises the question if higher secretion rates at faster growth are primarily attributed to a higher fraction of cells in G_2/M phase or rather to metabolic rates and other growth-associated effects.

To differentiate between effects of the cell cycle and the growth rate on global transcriptional regulation patterns, we established a method for the sorting of fixed cells according to their DNA content and subsequent isolation of RNA suitable for microarray analysis. This method was then applied on HSA-secreting *P. pastoris* cultivated in glucose limited chemostat cultures at a slow and a fast growth rate setpoint, yielding insights into the interplay of cell-cycle- and growth-rate-specific gene regulation and potential implications regarding protein production.

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P21-2

Optimal fermenting condition for the production of immunostimulating polysaccharides in Korean traditional rice wine

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Korean traditional rice wine (*Mageoli*), which contains 6–7% (V/V) alcohol, is a popular alcoholic beverage in Korea. It is brewed using '*Nuruk*' or *koji* as a starter, which is steamed rice fermented with yeasts, in a 2-step process: saccharification and alcohol fermentation. In this study, we investigated the effect of crude polysaccharides produced by *Mageoli* (MCP) fermentation using four yeast strains, various temperatures, and fermenting periods on the activation of macrophages, indicated by the change in levels of nitric oxide, IL-6, and IL-12. We also studied the chemical composition of the MCP. The highest concentrations of nitric oxide, IL-6, and IL-12 were observed in the peritoneal macrophage of MCP fermented with *Saccharomyces cerevisae* 113-4. For MCP obtained after fermentation at 25 °C, NO concentration increased 2.7–3.3 times, whereas IL-6 concentration 5.7 times, compared to another temperature condition. NO production on treatment with MCP from *Mageoli* fermenting for 5 days was the highest, increasing 2.2 times compared to that by 0-day MCP treatment. The results showed that the fermenting conditions resulting in the highest activity were observed in MCP inoculated with *S. cerevisiae* 113-4 and fermented at 25 °C for 5 days. Under these conditions, the fermented rice wine of functional MCP contained 78.6% neutral sugars, comprising mainly mannose, glucose, and galactose; 11.6% acidic polysaccharide; and 9.8% protein.

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P21-3

Analysis of genome-wide transcriptome of an amylolytic yeast *Saccharomycopsis fibuligera*

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An amylolytic yeast *Saccharomycopsis fibuligera* MBY1320, which was isolated from *nuruk*, exhibits raw starch-degrading activity. Transcriptomes of *S. fibuligera* MBY1320 grown in glucose and soluble starch as carbon source were analyzed. A total 80,245,884 reads were assembled into 7266 transcripts. Comparison of transcriptome indicated that 443 genes were differentially expressed, of which 228 genes were up-regulated and 215 genes were down-regulated. When grown in starch medium, expression of putative acidic protease was significantly decreased and that of alpha-glucosidase was increased. This study will provide the basis for understanding of the starch-degrading response of *S. fibuligera* MBY1320.

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P21-4

Biotechnological potential of highly specific proteolytic enzymes produced by micromycetes of *Aspergillus* genus

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The prevalence and danger of such potentionally fatal side effects as thrombosis are the reason for biotechnology to search for the new compounds for early diagnostics and treatment of such disorders. Nowadays medicine prefers enzymes with the animal origin, which are not always effective enough, but, moreover, are quite expensive. In this research, we analyzed the variety of activities of proteolytic enzymes, isolated from different species of the genus *Aspergillus*, which respects to various components of the hemostasis system.

The aim of this work was to identify the activity and specificity of proteases, produced by micromycetes *Aspergillus flavus, A. oryzae, A. sydowii* and *A.ustus.*

In this work, we used lyophilized preparations of extracellular proteases of microscopic fungi of the genus *Aspergillus.* The preparations were separated by isoelectrofocusing. The activities were measured with the specific chromogenic substrates of proteases of the hemostasis system.

It was demonstrated that *Aspergillus flavus* can be characterized by the presence of high activity, respected to plasmin and thrombin (342.59 and 444.44 units/mg of protein $\times 10^{-3}$ (U)), also it shows high activator activities to t-PA(476.85 U). For *A. oryzae* activator activity to protein C was detected (22.95 U). *A. sydowii* demonstrated plasmin-like activity (202.U). For *A. ustus* activities to thrombin(113.36 U) and Xa-factor(77.55 U) were demonstrated.

In conclusion, it should be stated, that fungi of the genus *Aspergillus* could be the potential producers of proteases with different activities to proteins of the hemostasis system. They can be used for diagnosis, as well as can find the therapeutic usage.

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P21-5

Metabolic flux analysis of glucose/ethanol metabolism in *Saccharomyces cerevisiae* cultures

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Many different cell factories are used for the production of biopharmaceuticals, among them *Saccharomyces cerevisiae* offers high volumetric productivity with low production cost being used for production of several largescale products, such as human transferrin and human serum albumin.

Conventional metabolism of *S. cerevisiae* is characterized by the consumption of large quantities of glucose and high production of ethanol, a by-product widely reported as inhibitor of cell growth. This metabolic behavior which describes the production of anaerobic by-products during aerobic fermentation is known as the Crabtree effect.

In order to achieve high productivity and product yields redirection of metabolic pathways is required. Thus, cell metabolism should be studied with the aim of finding the metabolic bottlenecks susceptible of modification. This optimization can be done by means of external operational strategies and/or by genetic modifications of enzymes related to the metabolic pathways.

In order to achieve high cell density for improving recombinant protein production, two different approaches can be implemented: enhancing biomass/glucose yield and decreasing generation of ethanol as a toxic by-product. In *S. cerevisiae* cultures, we have observed that under certain culture conditions, which are related to glucose concentration of the media, biomass/glucose yield increased and ethanol production decreased while maintaining similar growth rate.

The objective of this work is to evaluate the redistribution of the main metabolic pathways of *S. cerevisiae* that could explain the behavior described. For this reason, a metabolic model that allow to quantitatively describe the flux distributions of the cellular metabolic network is analyzed.

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P21-6

Ergot alkaloid production in Claviceps purpurea is regulated by tryptophan related genes

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Claviceps purpurea is a phytopathogenic fungus predominantly causing ergot disease of rye and triticale. The *Claviceps* sclerotium contains a high concentration of ergot alkaloids (EA) which have a broad pharmaceutical use. There is a continual effort for mutants of *C. purpurea* with increased EA content.

The basic building blocks for EA are the dimethylallyl pyrophosphate (DMAPP) and the aromatic amino acid L-Tryptophan (Trp). We have focused on anthranilate synthase (AS), a key enzyme in the conversion of anthranilate to tryptophan, and dimethylallyltryptophan synthase (DMATS), a key enzyme in ergot alkaloid biosynthesis. Both enzymes are regulated by Trp. In the case of AS Trp works as an allosteric feedback inhibitor, in the case of DMATS Trp serves not only as a substrate but also as an allosteric activator. Recently it has been shown in various plants and in filamentous fungus *Aspergillus fumigatus*, that expression of mutated AS form (Trp feedback resistant) led to increased Trp pool, moreover in the fungus it also enhanced production of Trp-based secondary metabolites. With respect to DMATS, this is the first attempt to overexpress this enzyme in *Claviceps purpurea*. Our progress will be discussed.

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P21-7

From wheat straw to xylitol: bioconversion by a genetically engineered *Candida guilliermondii* strain

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Wheat straw is a major crop in Europe and represents a promising raw material for the production of high value products. One of these products is xylitol, a sugar alcohol commonly used as a sweetener in food and also pharmaceuticals. In order to produce xylitol from wheat, two essential processes play a crucial role: (1) biomass pretreatment and (2) microbial fermentation. Biomass pretreatment (e.g. Steam Explosion) is necessary to obtain a sugar solution for the microbial fermentation. This process also releases metabolic inhibitors that drastically reduce the efficiency of the subsequent fermentation step. In our laboratory, we aim to use the yeast *Candida guilliermondii* for the production of xylitol in a natural manner. This is considered as a sustainable production platform to produce xylitol in an environmentally friendly manner. In this work, we engineered the xylitol metabolism of *C. guilliermondii* by modulating the activity of the two main enzymes involved in this process, xylose reductase and xylitol dehydrogenase.

Our results suggest, that the described strategy is successful in modulating the activity of these enzymes and the xylitol metabolism. Additionally, we provide a descriptive analysis of *C. guilliermondii* tolerance towards the main growth inhibitors released during the pretreatment of wheat straw, which will facilitate the optimization of the whole process.

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P21-8

Construction of a tool for multiple gene over-expression though integrative transformation for primitive filamentous fungus *Ashbya gossypii*

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Ashbya gossypii is a flavogenic filamentous fungus employed successfully for industrial riboflavin (Vitamin B_2) production. It possess the smallest known eukaryotic genome with high level of gene synteny with *Saccharomyces cerevisiae* genome. Full genome sequencing and the ease of genetic engineering made *A. gossypii* a model organism for metabolic engineering as well as for studying fungal developmental biology. It has been recently explored for production of single cell oil, saturated fatty acids, recombinant

proteins and flavor compounds. We have developed an easy tool for multiple gene overexpression in *A. gossypii*. This tool in the form of vector–pUG6-GPDp is made by cloning an open AgGPD (Glyceraldehyde 3-Phosphate Dehydrogenase) promoter in proximity to G418 antibiotic resistance marker gene flanked by loxP sites. The cloned vector can be used for synthesis of the cassettes simply by PCR for any gene overexpression. Linear cassette can be transformed to *A. gossypii* which replace native targeted gene promoter with constitutively expressed GPD gene promoter through recombinase system. Genome modification through integrative transformation provide the great advantage of stability of transformed strains. Moreover, loxP sites aid in removal of the selection marker gene G418 by transient expression of Cre-recombinase after successful selection of clones. This allows the same tool to be used with same marker gene for second gene over-expression. We are targeting over-expression of FMN1 gene (Riboflavin Kinase) and ZWF1 gene (Glucose 6-Phosphate Dehydrogenase) with the cassettes made from the vector pUG6-GPDp for enhanced flavins production.

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P22-2

Strawberry polyphenols with antihypertensive and antioxidant activities as natural antifungal in strawberry-orange juice

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Tucumán is the leading producer of strawberries in Argentina and consumption of strawberry juice is high. The contaminating microbiota that can be present in strawberry juice is mainly constituted of yeasts extraordinarily resistant to chemical preservatives commonly used in the juice industry. On the other hand, there is a high amount of damaged strawberries which is discarded during harvest, so that their reutilization as source of polyphenols with antifungal activity could be a good alternative.

The aim of this work was the recovery and identification of phenolic compounds present in strawberries and the investigation of their beneficial properties, such as antimicrobial, antihypertensive and antioxidant activities. Orange-strawberry juice was used as food model system. Our results demonstrated that strawberry polyphenols possess antioxidant and antihypertensive activities up to 60%. Microbiological and sensorial analysis of juices enriched with polyphenols and inoculated with yeasts were performed throughout two weeks of storage at 4, 20 and 28 °C. The addition of phenolic compounds significantly increased antioxidant and antihypertensive capacity of strawberry-orange juice when compared to fresh control. Polyphenols treatments, alone or combined with metabisulfite, completely killedspoilage yeasts in juice samples, at all conditions tested. Thus, polyphenols from strawberry could be feasible alternatives to improve microbiological quality with low impact on the organoleptic properties of polyphenols-enriched strawberry-orange juice.

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P22-3

In vitro antibacterial activities of the bark and leaf extracts of *Anacardium humile* (St.) Hil against multidrug resistant strains

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Anacardium humile (St.) Hil is a plant found throughout Brazilian Cerrado biome. This study evaluated the antibacterial activity of *Anacardium humile* bark and leaf extracts against multidrug-resistant bacteria isolated from diabetic foot infections (DFI). The activity was determined by agar disk-diffusion (DD), broth microdilution (BD), checkerboard, and time-kill methods. By DD and BB methods, leaf and bark extracts inhibited the growth of gram-positive (BD, bark: $n = 40$, 83.33%, leaf $n = 38$, 79.16%; DD, bark:: $n = 32$, 66.66%, leaf: $n = 28$, 58.33%) and gram-negative bacteria (BD, bark: $n = 20$, 76.92%; leaf: $n = 20$, 76.92%; DD, bark: $n = 16$, 61.53%, leaf: $n = 16$, 61.53%); including bacteria commonly found in DFI, such as *Staphylococcus aureus*, *Streptococcus agalatiae*, *S.*

pneumoniae, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus*. By checkerboard method, the combination of bark extract (35%) or leaf (40%) with Cefoxitin on Gram-negative bacteria had similar synergistic effects while the leaf extract (45%) showed greater synergistic effect than bark extract (17.5%) in combination with Amoxycillin/Clavulanic Acid on gram-positive bacteria. The time-kill kinetic profile showed bactericidal activity with dose and time-dependent properties, suggesting that bark and leaf extracts may potentiate the effects of antibiotics. Our data suggest that extracts of *Anacardium humile* should be considered an alternative source of research for antibacterial agents acting on gram-positive and Gram-negative multidrug-resistant bacteria. However, the isolation of bioactive compounds and additional studies should be performed to understand the mechanisms of bactericidal action and define its real efficacy and toxicity.

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P22-4

Antimicrobial activity and fatty acids composition of actinobacteria isolated from Siberian caves

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Extreme and unusual ecosystems such as isolated ancient caves are considered as potential tools for discovery of novel natural products with biological activities. Actinobacteria that inhabit these unusual ecosystems are examined as a promising source for development of new drugs. In this study we focused on the preliminary estimation of fatty acids composition and antibacterial properties of culturable actinobacteria isolated from water surface of underground lakes located in Badzheyskaya and Okhotnichya caves in Siberia. Here, we present isolation of 17 strains of actinobacteria that belong to *Streptomyces*, *Nocardia* and *Nocardiopsis* genera. Using antibacterial assay against bacteria and fungi we found that a number of strains of genus *Streptomyces* isolated from Badzheyskaya cave demonstrated inhibition activity against bacteria and fungi. It was shown that representatives of genera *Nocardia* and *Nocardiopsis* isolated from Okhotnichya cave did not demonstrate any tested antibiotic properties. However, despite the lack of antimicrobial and fungicidal activity of *Nocardia* extracts, those strains are exceptional in terms of their fatty acids spectrum.

When assessing fatty acids profile, we found that polyunsaturated fatty acids were quantitatively dominant in extracts of *Nocardia* sp. and *Streptomyces* sp. grown in different media. Saturated fatty acids were the second most abundant type in the fatty acids profile. It was due to palmitic acid. Also, a few monounsaturated fatty acids were detected. The obtained materials can become a basis for development of approaches to use bacteria isolated from caves as a biological source of biologically active compounds to create medical and veterinary drugs.

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P22-5

The effect of the addition of fresh and dried starter cultures on microbiological and chemical parameters of a smoked sausage "Alheira"

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Lactic Acid Bacteria (LAB) and their bacteriocins can be successfully used as natural preservatives in meat products. This work aimed to investigate the effect of fresh and dried starter cultures of an autochthonous bacteriocinogenic LAB strain (*Lactobacillus plantarum* ST153Ch) on the physicochemical and microbiological characteristics of "Alheira", a traditional Portuguese smoked product. "Alheira" with the addition of fresh (ca. 10^8 cfu/g) and dried (ca. 10^7 cfu/g) culture and "Alheira" control (no starter culture added) were produced by an industrial meat company. The antilisterial activity of this culture in this food matrix was also investigated, with some samples being inoculated with *Listeria monocytogenes* (ca. 10^7 cfu/g) in a lab. Humidity, pH, water activity (aw), acidity, peroxide index, detection of *L. monocytogenes*, *Salmonella* spp., sulphite reducing clostridia, *Yersinia enterocolitica* and enumeration of *L. monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Enterobacteriaceae*, lactic acid bacteria (LAB), yeasts and moulds were performed at 0, 15, 30, 45 and 60 days of storage at 4 °C, according to ISO methodologies. Pathogenic and indicator organisms were not detected or were below acceptable levels in all samples. In all samples, LAB counts increased during storage and reached similar values after 60 days (ca. 10^{11} cfu/g). Both fresh and dried cultures inhibited growth of *L. monocytogenes* during storage. Significant differences in moisture content between control and inoculated samples were found along storage. Values of pH and acidity index decreased and peroxide index increased up to 45 days of storage in inoculated samples. Overall fresh and dried cultures showed similar performances.

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P22-6

A 3D *in vitro* Human Airway Epithelial platform for the development of novel anti-bacterial and antiviral drugs

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Respiratory bacterial and viral infections cause frequently mild to severe diseases worldwide, it is a serious public health problem. To develop new anti-bacterial and viral drugs, new relevant and efficient tools are needed. We report herein a platform of 3D epithelia made of human primary airway epithelial cells, MucilAir™, for anti-bacterial and viral drug screening.

As proof-of-concept, typical disease-causing bacteria and viruses were used to infect MucilAir™ tissues. The effect of bacterial and viral infections can be easily and accurately monitored with several endpoints: Trans Epithelial Electrical Resistance (TEER); cytotoxicity (LDH), cilia activity, mucin and IL-8 release.

Pseudomonas aeruginosa (PA) infection induces a loss of TEER, 20% cytotoxicity and an increase of Il-8 (+100 ng/ml). On the contrary, SP strongly increases the mucin production. Antibiotics like Meronem efficient inhibits the bacterial growth and abrogates the bacterial effect on endpoints.

Clinically relevant Rhinovirus (A16, C15), Enterovirus (EV68) and Influenza A virus (H1N1, H3N2) strains were added to fully differentiated MucilAir™ – Pool. Released viral genome copy number, overall mucin secretion, cilia beating frequency, MCC and tissue integrity were assessed daily during 4 days. MucilAir™ supports high rate of replication for all tested viruses, including difficult-to-culture HRV-C15. Anti-viral drug, Rupintrivir, efficiently inhibited the replication of HRV-A16 and HRV-C15 in a dose and time dependent manner and restored MCC impaired by EV68. Oseltamivir reduced the replication of H1N1 and H3N2 and restored the impaired barrier function.

These results demonstrate that MucilAir™ is a robust, reliable and relevant tool for anti-bacterial and antiviral drug development.

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P22-7

Taking first steps towards the installation of an innovative process chain for the discovery and production of new antimicrobials

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In times of a global increase of antibiotic resistances there is a pressing need for the development of innovative strategies for the discovery of new antibiotic substances. Since former strategies for antimicrobial drug discovery mainly focused on synthetic chemicals, modern approaches are now returning to the investigation of natural resources [1]. To approach this urgent task, we are establishing a laboratory for the isolation and production of new antimicrobial agents from natural products. The installation of this laboratory is realized within the interdisciplinary research project "AdvancedBioPro". The laboratory will eventually provide a complete bioprocess chain: from initiation and development to optimization and upscaling for industrial application. State-of-the-art technology is used to design an advanced robot-based discovery platform, including innovative high throughput screenings for the isolation of microorganisms as well as for antimicrobial activity and cytotoxicity testing.

We successfully accomplished the following first steps: A robot-based high throughput screening was developed to isolate bacteria from sludge and to test them for their antimicrobial activity against human pathogenic bacteria. A promising candidate, identified by MALDI Biotyper System as an *Escherichia coli* (*E. coli*) strain, was shown to exhibit antimicrobial activity against enteropathogenic *E. coli* (EPEC). The purification of the antimicrobial compound was achieved by combining different precipitation and chromatography steps. LC-MALDI-TOF MS-based analysis is performed to identify the purified substance. Additionally, a high throughput cell imaging technology is used to evaluate the cytotoxic potential of the purified substance. Further steps will comprise the fermentation development, large-scale production and purification of the product.

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P22-8

Unmasking the potential of three Colombian *Streptomyces* strains as antibiotic factories

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The need for new compounds to tackle antimicrobial resistance is driving the search for *Streptomyces* – the gram-positive soil actinomycetes that produce a wealth of specialised metabolites, including antimicrobials – in unique environments. Alongside this, genomic mining and elucidation of new molecules and related genes by heterologous expression stand out as promising alternatives to identify novel antibiotics. Here we present the first insights into the potential for new ones in three *Streptomyces* strains (*S. avidinii* CG885, *S. microflavus* CG893 and *S. subrutilis* C926) isolated from a high Andean ecosystem in Colombia. We provide evidence of that from a genomic mining approach and antimicrobial assays against ESKAPE pathogens. We also present findings on the gene clusters related to some of these likely novel molecules. We report analysis with the computational tool antiSMASH that led to the identification of 41 distinct clusters with apparent relation to antibiotics and less than 10% identity with other known clusters. In the antimicrobial assays, all ESKAPE pathogens, except *Pseudomonas aeruginosa* ATCC 27853, showed inhibition by at least one of the *Streptomyces* strains. *S. avidinii* CG885 presented the strongest antimicrobial activity through time, while the other two strains displayed changes after some days, suggesting instability of the compounds. Findings in this project provide evidence of the vast potential for novel antibiotics in these three *Streptomyces* strains and insights into their characteristics. These results highlight also the benefit of combining experimental work and genome mining approaches to study isolates from unexplored environments and identify new antimicrobials.

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P22-9

Phage-mediated transduction of sulfonamide resistance genes in *Microbacterium* spp.

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The mobilization and transfer of different antibiotic resistance genes by bacteriophages have been described for various bacterial species. Also metagenomic studies have shown a high content of bacterial genes and genetic elements in the viral communities, indicating that transduction (both specialized and generalized) frequently occur. However, phage-mediated transfer of sulfonamide resistance genes has never been documented. Here we present

transfer of the sulfonamide resistance gene *sul1* via bacteriophage transduction.

Methods: The transduction system consisted of a virulent environmental bacteriophage and two phage-susceptible *Microbacterium spp*. strains: a donor harboring *sul1* and a recipient lacking *sul1*. Experiments were carried out with different phage/bacteria ratios, both incubated in 25% Standard nutrient broth and Brunner mineral medium, respectively. Both media were supplemented with 1 mM sulfamethoxazole (SMX). Gene transfer was tested by PCR and primers specific for the target gene.

Results: Transductants were obtained only in low phage-bacteria ratios in nutrient-rich medium. Therefore, the transduction probability depends on multiplicity of infection (MOI) and nutrients supporting bacteria-phage growth. The spontaneous production of antibiotic resistant mutants as well as gene transfer by transformation could be excluded using appropriate controls.

Conclusions: It could be demonstrated that bacteriophages can contribute to the accumulation and propagation of antibiotic resistance genes. This study better understand the complex ecological role of bacteriophages in the environment.

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P22-10

Isolation and screening of microbial strains for potential antimicrobial activity

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Antimicrobial agents constitute a very promising research field. Considering that the most antibiotics in use are microbial products, there is a continuous need to find new reserves of biologically active compounds. Microorganisms have been extensively studied for their capacity to produce pharmaceutical compounds and they are still considered very attractive sources in this field. Bacillus and Actinomyces are well known for antibiotics production, polypeptides of low molecular weight, especiallys and also, in the recent years for lantibiotics biosynthesis, which have unique structural characteristics. Many scientists have focused on screening programs for microbial productions of antibiotics. The overall aims of this study were to isolate new microbial strains from different soil and honey samples, to select the most suitable culture media for conservation and morphological characterization and to screen the strains for antimicrobial activity. Some selected microorganisms, with antibiotic potential, were preliminary identified using mass-spectrometry. They belong to the genera *Bacillus* and *Streptomyces*. The preliminary screening for antibiotic production, showed good results against 2 human pathogens, *E. coli* ATCC 8739 and *S. aureus* ATCC 6538 strains (inhibition zones >40 mm).

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P22-11

Detection of bla_{NDM-1} gene among the carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates

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NDM1 has been reported form Nepal along with various other classes of carbapenemases like KPC and OXA-48.These enzymes make the treatment of infections impossible using carbapenems so only drug left is colistin. To defeat the problem it seems as a great necessity to focus the clinical researches on the study of emergence and spread of these organisms.

The objective of this research is to ascertain the carbapenemase production among carbapenem resistant *E. coli* and *K. pneumoniae* and then detecting the presence of bla_{NDM1} gene.

During the period of this study from November 2016 to April 2017, a total of 1503 samples were processed. Urine, blood, swab, pus and CSF samples were collected from the patients visiting Children's Hospital in Kathmandu. The samples were cultured in MacConkey Agar and Blood Agar for selection which were further cultured in Nutrient Agar. Carbapenem i.e. imipenem (82.2%) and meropenem (79.1%) were found to be most effective antibiotics against the isolates. 46.69% of the isolates were found to be MDR. From the pool of 94 *E. coli* and 35 *K. pneumoniae* isolated, 34 *E. coli* and 18 *K. pneumoniae* were screened as possible carbapenemase producers in this study. Two isolates of *E. coli* and 3 isolates of *K. pneumoniae* were confirmed as carbapenemase producers by Modified Hodge Test. Furthermore, PCR was carried out among them for the detection of blaNDM-1 gene. Genetic analysis of those MHT positive isolates showed 1 among 2 *E. coli*, 2 among 3 *K. pneumoniae* to be NDM-1 producers.

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P23-1

Comparative metagenomic analyses of sheep and cow rumen contents and their effect on methane production of anaerobic batch fermentation

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Nowadays, extensive usage of fossil fuels leads to serious environmental problems such as air, water or soil pollution and via greenhouse effect, it contributes to climate change. Since they are being exhausted, the non-renewable energy sources are available for only a very short time in the future. Therefore, finding novel, clean and renewable energy sources instead of fossil fuels is among the most urgent energetic challenges. Biogas is also produced by natural procedures including the digestive processes of ruminants. Consequently, the nature inspired biochemical routes might be exploited for enhanced biogas production.

In this study, we examined the microbial and chemical compositions of rumen contents of two different herbivores (cow and sheep) and their effects on methane yield in anaerobic digestive reactors. First of all, the genomic DNA samples from rumen contents were isolated and used for metagenomics analyses (complete shotgun and 16S rRNA metagenomic analyses). The raw data were analysed with CLC genomics workbench and MEGAN Software. Microbial communities of sheep and cow rumen were compared

to each other in order to identify microorganisms playing the most fundamental role in rumen fermentation.

Furthermore, the effects of rumen contents were also tested in biogas fermentation. Our experiments clearly proved that sheep rumen could substantially promote the biogas yield in commercially used biogas reactors.

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P23-2

Biotechnological production of sesquiterpene from glycerol

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Terpenes, the most available compounds naturally present in plants and their essential oils, are widely applied in aroma, biofuels and pharmaceutical industries. This work is focused on biotechnological production of a specific sesquiterpene ($C_{15}H_{24}$), known for potential anti-inflammatory properties and precursor of oxygenated derivative ($C_{15}H_{22}O$) with remarkable anti-carcinogenic activities. Fed-batch experiment using engineered *Escherichia coli* resulted in 0.54×10^{-3} kg sesquiterpene/kg broth at 33.4×10^{-3} kg biomass/kg broth after 62.8 h (2.26×10^{5} s) age using dodecane (6% v/v) for *in situ* product recovery. Terpene concentration increased along time, suggesting improvements for longer experiments.

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P23-3

Exploring the metabolic potential of oleaginous actinomycetes in biodiesel production from cassava wastewater

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The depletion in fossil fuel reserves has instigated the search for renewable sources such as biofuels. Biofuel production provides a sustainable alternative to fossil fuels. However, the progression of biofuel industry has been largely affected by several uncertainties including the sustainability of production processes. Finding an economically viable process and the right substrates have been a source of constant debate. Biodiesel production is one such area which is gaining momentum in the last few decades. We are currently investigating cassava wastewater as a substrate and also as a source of oleaginous bacteria. The production of tri-acyl glycerol from mycolic acid containing actinomycetes predominantly Rhodococcus, has been identified as a potential source. These bacteria which are largely ubiquitous provide a significant amount of triglyceride when cultivated under low cost waste. The growth of oleaginous bacteria for the accumulation of triglycerides on low cost and abundant, cassava waste still remains unexplored, especially in Nigeria which is the largest producer of cassava in the world. We started our initial investigations on the cassava waste water sample following culture dependent and independent approach. We prognosticate that the identification of microflora isolated from different stages in the sample will provide a basis for understanding the nature of the substrate and the potential for the synthesis of biodiesel from cassava waste water.

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P23-4

Oil extraction from the oleaginous yeast *Schwanniomyces occidentalis*

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Microbial oil can be produced in a green and sustainable way using the oleaginous yeast *Schwanniomyces occidentalis*. This yeast can reach a lipid content of 41.9% (g/g, DCW) using a broad spectrum of C5 and C6 sugars under nitrogen-limited conditions. A drawback of using oleaginous yeasts is that the extraction of lipids is difficult since they are stored as intracellular oil droplets. Various extraction methods have been tested that are categorized into chemical, thermal, mechanical or biological methods. However, most of these methods are economically or energetically unfavourable. The most promising extraction methods are based on wet biomass, since it discards the highly unfavorable drying step. Industry currently use mechanical high-pressure homogenization cell disruption protocols. Biological methods using enzymes are promising, since this is a non-destructive method that can prevent thermal degradation of compounds. Here, we describe a disruption method using a tailor-made enzyme cocktail that can be applied to the wet biomass content of *S. occidentalis*.

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P23-5

Manually modified design of experiments for early biocatalyst evaluation – lipase-catalysed fatty acid methyl ester production

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Lipase-catalysed fatty acid methyl ester (FAME) synthesis is a promising alternative for biodiesel production. Rapid screening, selection, and evaluation of different lipase candidates with varying properties are critical tasks during early biocatalyst development. Conventional approaches employ statistically designed experimentation in order to elucidate the effects of reaction conditions on new biocatalyst candidates. Most commonly, central composite designs (CCDs) are used whose structures are defined by the number of factors tested along with their operational value ranges. In early stages of biocatalyst development, these ranges may not be accurately identified due to lack of information about physico-chemical properties of the reaction system such as solubility limit. As such information is acquired, initial experimental design may need to be modified. In this case, rather than discarding the already-performed experiments, a manually modified experimental design may be used.

In this study, FAME production from waste cooking oil, via a novel, organic solvent-tolerant Cryptococcus diffluens D44 lipase-catalysed transesterification reaction is evaluated using a manually curated, CCD-based experimental design. The reaction conditions studied are reaction temperature, enzyme concentration, and alcohol-to-oil molar ratio. Product titre and productivity are responses.

Due to the solubility limit of the lyophilised crude enzyme used, experimental points in the three-dimensional design space related

to enzyme concentration had to be modified from the original CCD. Main effects plots, generated using the modified CCD, gave reliable enough results to allow a rapid and early evaluation of the new biocatalyst candidate for FAME production.

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P23-6

Optimization of lipid accumulation in thermotolerant yeast *Candida* sp. Sbc 06 using molasses as carbon source

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The demand of biodiesel for using as alternative energy source has increased continuously. Microbial oil could be more attractive for biodiesel production if it production cost is low. Cheap substrate and low energy consumption process could solve this problem. Molasses, a waste from sugar production, and thermotolerant oleaginous yeast which could be able to grow without controlling temperature were used in this study. Thermotolerant oleaginous yeast *Candida* sp. Sbc 06 was isolated from the soy bean cake, a waste from soya milk production, and could utilize sucrose as a carbon source. Response Surface Methodology (RSM) was used to optimize conditions for oil production from molasses at high temperature by this oleaginous yeast. Inoculums size, total sugar and nitrogen concentrations were optimized by using the Box–Behnken method. All cultures were incubated at 40 °C and 200 rpm for 6 days. The model showed the significant result which implied that the designed factors had a significant effect on lipid accumulation. Results indicated that total sugar and nitrogen concentrations had statistically significant ($P \leq 0.05$) influences on single cell oil production. From the Design Expert program, the obtained inoculums size, nitrogen source and total sugar concentrations were 6.22, 1.43 g/l and 94.77%, respectively, which the highest obtained lipid content was 69.5%. Thermotolerant oleaginous yeasts *Candida* sp. Sbc 06, thus, could be an alternative potential single cell oil production strain for biodiesel production from molasses.

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P23-7

Biohythane production from co-digestion of palm oil mill effluent with biomass residues of palm oil mill industry

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The solid waste residues from oil palm industry such as decanter cake (DC), oil palm trunk (OPT), oil palm frond (OPF), empty fruit bunch (EFB) was co-digested with palm mill oil effluent (POME) for enhanced volumetric of biohythane production via thermophilic two-stage reactor. POME gave higher hydrogen yield (50 mL-H_2/gVS) when compare with other biomass such as DC, FB, OPT, EFB and OPF. POME co-digestion with 5%FB gave the high hydrogen yield (56 mL-H_2/gVS) than POME alone. In the second stage, methane was produced from organic matter in the effluent of hydrogen reactor. The high content of VFA in 20%DC was a good substrate for methane production. Co-digestion POME with biomass residues has potential to improve methane yield with 321 mL-CH_4/gVS from 10%OPT higher than methane yield from POME alone (138 mL-CH_4/gVS). All the co-digestion POME with biomass residues in the first stage has lower hydrogen yield when compare with yields from the sole substrate, while co-digestion increased the methane yield in the second stage. The results of the biochemical hydrogen potential (BHP) test indicate an antagonistic between two substrates has a competitive effect in the final production. While synergistic effect appeared in methane stage except POME co-digestion with DC5%, DC20% and FB15% gave the antagonistic effect. Mixed hydrogen and methane (biohythane) has a synergistic effect in POME co-digestion with DC5% with energy yield of 1.09 MJ/gCOD. During two-stage hydrogen and methane production *Clostridium* sp. was dominated in hydrogen stage and change to *Methanoshaera* sp. in methane stage.

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P23-8

Reuse of the effluent from the dark anaerobic fermentation of biohydrogen production for microalgae lipid accumulation

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Dark fermentation is an effective way to produce biohydrogen. Besides biohydrogen, organic acids such as acetic acid and butyric acid are present in the effluent; however, the effluent is often regarded as waste and can cause considerable environment pollutions. Therefore, this study aims to reuse the effluent from biohydrogen fermentation to reduce waste and to increase valuable products from the bioprocess. Mixed-culture hydrogen-producing bacteria, *Clostridium butyricum* CGS5, *Clostridium pasteurianum* CH_4, and *Klebsiella* sp. HE1, produced an effluent containing formic acid (49.1 ± 2.4 mM), butyric acid (36.2 ± 6.6 mM), acetic acid (36.8 ± 3.8 mM), and propionic acid (11.9 ± 5.7 mM). These organic acids are reported as possible carbon sources or stimulants for microalgae lipid accumulation. A potential lipid production microalgae strain, *Scenedesmus abundans*, was treated with two methods to accumulate cellular lipids: nitrogen starvation and biohydrogen fermentation effluent addition. Nitrogen starvation produced a dry weight of biomass of 724 mg/L and a lipid abundance of 36.0 wt% while the addition of fermentation effluent produced a dry weight of biomass of 508 mg/L and a lipid abundance of 33.7 wt%. These results show that the biohydrogen fermentation effluent had a similar effect as the nitrogen starvation for increasing the cellular lipids amount in *Scenedesmus abundans*. We are currently testing the effects of the fermentation effluent on other microalgae strains and analyzing the organic acid composition along microalgae lipid accumulation to understand the carbon flow during the process.

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P23-9

Production of valuable carbon containing products with a new module-based vector system in cyanobacteria

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Regarding climate change, the reduction of CO_2 emissions became one of the major topics in the last few years. On the one hand, photosynthetically active organisms, like cyanobacteria, offer a great possibility to reduce CO_2 emissions by using photosynthesis and to synthesize various biofuels and chemicals by genetic engineering. On the other hand, this strategy leads to a reduction of the dependency on petrol-based fuels in our society. Gao et al. (2012, 2016) showed enhanced ethanol production up to 5.5 g L^{-1} by using the genetically engineered cyanobacteria *Synechocystis sp*. PCC6803. Based on these improvements, strategies for obtaining higher cell densities and further enhanced ethanol yield were developed. Therefore, a new module-based expression vector system with the possibility for easy exchange of DNA fragments, such as homologous integration sites, promoters and genes, was designed and will be presented. For ethanol production, the genes encoding alcohol dehydrogenase and pyruvate decarboxylase will be integrated into the cyanobacterial genome. Constitutive as well as inducible promoters will be tested for maximum ethanol production in *Synechocystis sp.* PCC6803 in a single or a two-step cultivation process. In further approaches, the exchange of homologous sequences for the knock-out of alternative pyruvate consuming pathways and, therefore, the redirection of pyruvate into the ethanol pathway will be performed. Strategies and gene constructs for high ethanol production in *Synechocystis sp.* PCC6803 will be presented.

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P23-10

Biomethanation of CO_2 with electrolytic hydrogen by hydrogenotrophic methanogens

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Biogas produced by anaerobic fermentation of waste water, sludge and organic wastes is the significant renewable source of energy. It contains, next to methane, also unavoidable amount of CO_2. An energy contained in methane, particularly its heat component, has not been fully utilized everywhere yet. On the contrary, solar or wind powers produce electrical energy independently on a current consumption. The surplus electricity then causes problems with the overloading of the distribution grid. It can be stored in the form of H_2 produced by the electrolysis of water. The energy of hydrogen can be converted to the easily utilizable and transportable form by biological methanation of CO_2 from biogas to biomethane using the activity of hydrogenotrophic methanogens. These Archae are able to catalyzed biological conversion of CO_2 and electrolytic H_2 to biomethane at temperatures of 35 or 55 °C and pressure close to atmospheric. Biomethanization can take place directly in anaerobic digesters or in separate bioreactor. The main goal of our project is to find the optimal conditions of biomethanation in terms of process parameters. Mesophilic and thermophilic reactors were operated with CO_2 and H_2 as substrates to enrichment of anaerobic consorcium by hydrogenotrophic methanogens. The efficiency of hydrogen utilization was the main problem in the process because of the low gas–liquid mass transfer of H_2. Therefore, the method of effective input of hydrogen into the system, have to be optimized. Possibilities of implementation of this method of biogas upgrading directly into biogas plants are promising.

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P23-11

Co-production of polyhydroxyalkanoates and carotenoids by *Paracoccus sp.* strain LL1

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Polyhydroxyalkanoates (PHA) have emerged as a potential alternative to synthetic plastics, although mass production is limited by the cost incurred in feed materials. Co-production of various valuable bioproducts with PHA has been proposed to reduce the overall production cost. Here, high yield co-production of PHA and carotenoids was achieved in single fermentation by *Paracoccus* sp. LL1. The halophilic bacterial strain could metabolize various substrates such as methanol, lactose, galactose, glycerol, fructose, mannitol, and valerate. Under batch fermentation using mineral media supplemented with 2% glycerol, *Paracoccus* sp. LL1 produced 3.77 g/L PHA and 3.6 mg/L of carotenoids after 96 h. A 2.2 fold increase in total dry cell weight (DCW) was achieved through cell retention culture of *Paracoccus* sp. LL1, resulting in maximum DCW (24.2 g/L) containing 39.3% PHA and improved total carotenoid production. The highest carotenoid concentration of 12 mg/L was obtained using 1% valerate as the carbon source. The type of co-produced PHA was poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer with a high 3-hydroxyvalerate fraction (95%).

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P23-12

An update on the large-scale microalgal ocean cultures for sustainable biofuels production in Korea

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Most of the culture systems today may not be suitable for biofuel production economically. One of the possible solutions to overcome some of the challenges in microalgal mass cultures is the ocean culture system. A large-scale floating ocean cultures have several benefits: (i) lower CAPEX; (ii) no freshwater usage; (iii) relatively abundant seawater; (iv) ability to exploit the lower nutrient concentration in seawater; (v) no need to worry about evaporation; (vi) larger area to deploy; and so on.

We have reported the world first ocean test-beds for large-scale microalgal cultures in previous meeting. For the past two years, we have improved ion permeability of Selectively-Permeable Materials (SPMs) over 10 times by various means and the overall biomass productivity increased proportionally. The average biomass productivity of 20 g/m^2/day using just seawater on our ocean test-beds was achieved. Recent progresses and other remaining challenges for economical and sustainable marine microalgal biomass/biofuels production will be discussed based on our experience in Korea.

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P23-13

Optimization of acid pretreatment and bio-hydrogen production from *Chlorella sp.* biomass

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Microalgal biomass has emerged as a promising feedstock for bioenergy production. However, due to the strong cell wall of microalgae, pretreatment is needed to increase the biodegradability of the biomass and, therefore, its bioenergy potential. In this study, acid (H_2SO_4) pretreatment was used to solubilize *Chlorella* sp. biomass for bio-hydrogen production. The conditions for the pretreatment, i.e. acid concentration, pretreatment time, and biomass concentration, were optimized. Maximal glucose yield (10.2 mg/g-biomass) and pretreatment efficiency (2.78 g-glucose/g-inhibitor) were attained at 4% (v/v) H_2SO_4, 150 min of pretreatment time, and 40 g/L of the biomass. The levels of substrate concentration, substrate to inoculum (S/I) ratio, and initial pH were subsequently optimized for dark fermentative hydrogen production. The optimal conditions were 15 g-volatile solid (VS)/L, S/I ratio of 2 g-VS/g-VS, and initial pH of 5.5, under which maximal hydrogen production and hydrogen production rate of 26.3 mL/g-VS and 19.8 mL/L h, respectively, were attained.

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P23-14

Methane production from co-digestion of microalgae biomass with crude glycerol by anaerobic mixed cultures

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Optimization of factor affecting methane production from co-digestion of crude glycerol with algal biomass was investigate using response surface methodology (RSM) with central composite design (CCD). Investigated parameters used were crude glycerol concentration, inoculum concentration and algal biomass concentration. Inoculum concentration and algal biomass concentration had a significant individual effect on the methane production (MP) ($p < 0.05$). The interactive effect on MP was found between crude glycerol concentration and algal biomass concentration ($p < 0.05$). The optimal conditions were 20.02 g-VS/L of crude glycerol, 9.76 g-VS/L of inoculum concentration and 5.50 g/L of algal biomass which gave the maximum MP of 68.94 mL-CH_4/L. The difference between observed MP (58.88 mL-CH_4/L) and predicted MP was 14.59%. Using the optimum conditions, MP from co-digestion of crude glycerol with algal biomass was higher than control (crude glycerol without algal biomass), indicating a significant enhancement of MP by algal biomass. The polymerase chain reaction-denaturing gradient gel electrophoresis analysis indicated that the methane producers present in the fermentation broth was *Methanoculleus* sp. *Methanospirillum* sp. *Methanoregula* sp. *Methanosarcina* sp. and *Methanocaldococcus* sp., respectively.

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P23-15

Impact of xylose transporters on the improved glucose/xylose utilization and butanol production in *Clostridium acetobutylicum* HOL1

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Lignocellulosic biomass is the most abundant and renewable organic material in the biosphere. Using lignocellulose as a feedstock to produce biofuels and commodity chemicals is of economic and environmental significance. However, many problems must be solved before such ideal feedstock can be processed efficiently through biological routes. The sugar streams produced upon hydrolysis of lignocellulose are mixtures of hexoses and pentoses, mostly glucose and xylose. Unless both the glucose and xylose are utilized, the economics of converting lignocellulosic biomass into bio-based products are unfavorable. Most butanol-producing strains of Clostridium prefer glucose over xylose, leading to a slower butanol production from lignocellulose hydrolysates. It is therefore beneficial to find and use a strain that can simultaneously use both glucose and xylose. In this study, three xylose utilize-related transporters in *Clostridium acetobutylicum* HOL1 strain were overexpressed, individually. The higher xylose consumption were observed. Compared to the parental strain, the engineered strain produced more butanol from glucose and xylose simultaneously, at a higher xylose utilization rate and efficiency, resulting in a higher butanol productivity and yield. With different initial glucose: xylose ratios, glucose and xylose were consumed simultaneously at rates roughly proportional to their individual concentrations in the medium, leading to complete utilization of both sugars at the same time. Transcriptional studies on the effect of glucose and xylose supplementation, suggests the glucose inhibition on xylose metabolism-related genes were improved. The engineered strains could be good candidates for providing an economical process for butanol production from lignocellulosic biomass.

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P23-16

Scenedesmus dimorphus NT8c conversion of sugarcane bagasse hydrolysate into biodiesel

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Fossils fuels are the main world energy supply. Overuse of fossil fuel to fulfill energy requirement has generated environmental issues like air pollution leading to global warming. Increasing environmental problems and exhaustion of fossil resources has forced the researchers to explore alternative fuel sources. In this scenario biofuels are the promising alternative fuel to meet energy crisis. The aim of present study was to cultivate microalgae on lignocellulosic waste for biodiesel production. In the present study *Scenedesmus dimorphus* NT8c was cultivated photoautotrophically and mixotrophically on sugarcane bagasse hydrolysate. Green microalga was analyzed by studying its biochemical composition and growth parameters. *Scenedesmus dimorphus* NT8c cultures fed with sugarcane bagasse were found to have high growth rate,

maximum biomass productivity of 119.5 mg/L/D, protein contents of 34.82% and lipid contents as 15.41% as compared to the photoautotrophic cultivation. Microalgae grown mixotrophically are capable of photosynthesizing while metabolizing and assimilating organic carbon. Combination of these two metabolic pathways of autotrophic and mixotrophic cultivations can help in significant increase of biomass and lipid productivity upto 14.887 μg/ml/d. Microalgae are considered to be as sustainable and potential feedstock for biofuels. In addition, consumption of agri-industrial food wastes help to resolve issues of their dumping and pollution of natural environments

Keywords: Autotrophic; Mixotrophic; Scenedesmus dimorphus NT8c; FAME analysis; Photosynthetic pigments.

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P23-17

Fatty alcohols: enzymatically from free fatty acids

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Fragrance, food and transport industries could not imagine business without them: fatty alcohols [1]. Up-to-date, commercial production raises environmental concerns due to processes such as transesterification followed by hydrogenation or oligomerization of ethylene followed by oxidation [2]. Therefore, more sustainable routes such as microbiological systems are being explored since optimized bioprocesses are able to compete with chemical production [3]. In a two-step reduction process, enzymatic fatty alcohol synthesis can be achieved. In the first step, free fatty acid is reduced to fatty aldehyde via a carboxylic acid reductase (E.C.1.2.1.30, CAR). In the second step, fatty alcohol is being synthesised through endogenous aldehyde reductases present in *E.coli*. [4]. CAR mediated carboxylate reductions have been comprehensively studied in our lab [5,6], but little is known about the reduction of fatty aldehyde to alcohols via *E. coli* aldehyde reductases [2]. Towards optimizing titers and yields of fatty alcohol synthesis, 11 different *E. coli* strains have been tested for their potential of endogenous fatty aldehyde reduction after enzymatic conversion of fatty acid to fatty aldehyde via a CAR from *Neuropora crassa*. This knowledge will be useful in designing an efficient biocatalyst for production of fatty alcohols and analysis of the responsible genes for the endogenous aldehyde reduction.

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P23-18

Large-scale microalgal biomass production for hydrothermal liquefaction – petroleum refinery approach

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Sustainable microalgal biofuel is receiving much attention worldwide and to achieve a positive energy balance in the biofuel production, technological advances and high production systems will be required. In our present work, bio-crude production by hydrothermal liquefaction of microalgal biomass obtained from raceway pond cultivation was studied. Under optimal 30 cm culture depth and 65 rpm impeller speed, *Scenedesmus obliquus* was grown in open pond for studying the growth kinetics and lipid productivity over seasons. The biomass productivity during summer, rainy and winter seasons was estimated to be 11.15, 4.58 and 12.41 $g\,m^{-2}\,day^{-1}$ respectively and correspondingly, the lipid productivity was estimated to be 1.19, 0.42 and 1.30 $g\,m^{-2}\,day^{-1}$ respectively. The biomass slurry after harvesting was treated for hydrothermal liquefaction in a 2L reactor, at 300 °C temperature, 200 bar pressure, and 60 min residence time under both catalytic and non-catalytic conditions. In the absence of catalyst, a bio-crude yield of 35.1% was obtained. On an interesting note, bio-crude yield was increased from 35.1 to 45.1% by adding homogeneous acid catalyst CH3COOH, but an increase up to 40.2% was observed by the use of basic catalyst Na_2CO_3. Assessment of the bio-crude based on elemental composition and atomic ratio, was found comparable to the properties of petro-crude, which further implies its suitability in petroleum refinery for production of biofuel.

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P23-19

A redox fuel cell capable of converting biofuels to electricity at high rate

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The use of hydrogen peroxide in fuel cells has recently received increasing attention, primarily due to its several unique characteristics when compared with the use of gaseous oxygen. However, there are three issues associated with the use of hydrogen peroxide in fuel cells [1]. Firstly, the actual cathode potential is lower than the theoretical one, which is mainly attributed to the mixed potential resulting from the simultaneous hydrogen peroxide oxidation reaction on the cathode. Secondly, the hydrogen peroxide oxidation reaction releases gaseous oxygen, leading to a two-phase mass transport. Thirdly, the reduction of hydrogen peroxide in fuel cells has to use metal catalysts, such as platinum, palladium and gold [2].

In this work, we propose to create the cathode potential by introducing a redox couple to the cathode while to use hydrogen peroxide to chemically charge to redox ions. The redox cathode not only completely eliminates the mixed-potential problem associated with the direct reduction of hydrogen peroxide, but also enables a faster cathodic electrochemical kinetics even without noble metal catalysts [3]. It has been demonstrated that the direct ethanol fuel cell with a redox couple of V(IV)/V(V) yields a peak power density of 450 $mW\,cm^{-2}$ at 60 C, which is 87.5% higher than that of the conventional cell with direct reduction of hydrogen peroxide [4].

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P23-20

Zymomonas mobilis biofilm, a new challenge for ethanol production from lignocellulosic hydrolysate

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Zymomonas mobilis is an ethanologenic microbe that has been demonstrated to have a potential to be used in lignocellulose biorefineries for bioethanol production. *Z. mobilis* biofilm has previously exhibited high potential to enhance ethanol production by presenting a higher viable cell number, higher metabolic activity and higher resistance to toxic inhibitors than planktonic cells. *Z.mobilis* biofilm was approximately 2–3 folds more resistant to model toxic inhibitors from lignocellulosic hydrolysate (formic acid, acetic acid, furfural and 5-HMF) than planktonic cell. Biofilm has been found to be more resistant to these model inhibitors by physiologically up- regulate molecular chaperone DnaK and 50S ribosomal protein L2. *Z. mobilis* biofilm reactor was conducted using plastic composite support (PCS) as a carrier and regulated under repeated batch mode using rice straw hydrolysate as a substrate for the production of ethanol. Ethanol yield ($Y_{X/S}$) was maintained in the level of 0.36–0.38 g/g under the three repeated batches.

Keywords: Zymomonas mobilis, Biofilm, Toxic inhibitors, Ethanol, Up-regulated

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P23-21

Application of nutrient stress for enhanced carbohydrate accumulation in *Anabaena variabilis* for improved bioethanol production

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The energy crisis in the present era resulting from the insatiable demand for fossil fuel undoubtedly questions our future existence. Therefore, generation of a renewable alternative is the need of the hour. In this respect, the present investigation explores the suitability of a nitrogen-fixing cyanobacterium *Anabaena variabilis* as a feedstock for bioethanol production. Since carbohydrate serves as the main substrate for fermentation during bioethanol production, the primary focus of the study was to analyse the carbohydrate and its components in the biomass of the test cyanobacterium and strategize a protocol to enhance the cellular carbohydrate content by subjecting to nitrate and phosphate starvation. Under the control growth condition, the cyanobacterial species exhibited high innate carbohydrate content of 46.2% dcw (dry cell weight) constituted by 27.6% reducing sugar, 11.6% glycogen, 2.5% starch, 2.1% cellulose and a negligible amount of hemicellulose (1.2%). Although phosphate starvation resulted in a more profound increase in the cellular carbohydrate content (~1.4-fold rise compared to control) than nitrate starvation, the yield (mg L^{-1}) of the same was drastically affected due to decline in the biomass yield to 0.23 g L^{-1} from 0.57 g L^{-1} in control. Hence, to combat this situation, a two-phase strategy known as the biphasic-phosphate starved approach was adopted which resulted in maximal cellular carbohydrate accumulation of 63.1% dcw with its yield being ~1.3-fold higher than that of control. Consequently, the biphasic-phosphate starved culture generated the maximum bioethanol content of 28.2% dcw representing ~1.4-fold higher value than the control.

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P23-22

Outdoor cultivation of the green microalga *Chlorella minutissima* under fed-batch mode for biodiesel production

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Microalgae are considered as renewable feedstocks for biodiesel production. In the present study, we investigated the year round feasibility of a local isolate, *Chlorella minutissima*, under fed-batch cultivation mode for biodiesel production. Effect of culture depth (5, 10, 15 and 20 cm) on biomass and lipid yield of the open tank cultures was studied; various physicochemical parameters, viz. water temperature, dissolved oxygen concentration and pH were recorded. The maximum biomass and lipid yield were obtained at 15 cm culture depth. Experiments with low dose sequential phosphate addition (LDSPA) were also conducted at optimum culture depth. Under fed-batch cultivation, the average areal biomass and lipid productivities were 3.95 and 0.45 g m^{-2} day^{-1}, respectively, which can be projected to an annual biomass and lipid productivities of 13.02 and 1.47 tons ha^{-1} $year^{-1}$, respectively, considering 10 cultivation cycles $year^{-1}$. However, under LDSPA mode, the annual biomass and lipid productivities can be projected up to 15.4 and 2.29 tons ha^{-1} $year^{-1}$, respectively, thus demonstrating the superiority of LDSPA for higher lipid productivity. The biodiesel produced showed the predominance of monounsaturated and saturated fatty acid methyl esters. Moreover, the fuel properties were found to be comparable with various international and national standards of biodiesel, thus demonstrating its suitability as a substitute to fossil fuel.

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P23-23

Biohydrogen production from palm oil mill effluent in a continuous stirred tank reactor: effect of mixing speeds of Rushton turbine

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Production of biohydrogen from palm oil mill effluent (POME) was conducted in a 5 L continuous stirred tank reactor (CSTR) under thermophilic condition. The effect of mixing speeds of a Rushton turbine of the CSTR on the biohydrogen production was studied. The system was operated with the initial organic loading rate (OLR) of 55 gCOD Linfluent-1 d-1 and the hydraulic retention time (HRT) of 24 h. By varying the turbine speeds at 10, 50, 100 and 150 rpm, the process gave the hydrogen yields of approximately 2670, 2870, 3410 and 3480 mL H2 Linfluent-1, respectively. Since there was no significantly difference of the hydrogen yield, the turbine speed of 100 rpm was chosen. The continuously changing physical properties of the effluent suggested that the last two-third of the retention

time could be mixed at 10 rpm in order to minimize the input energy of the system.

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P23-24

Continuous two-stage anaerobic co-digestion of Skim Latex Serum (SLS) and *Rhizoclonium* sp. macro-algae for bio-hythane production

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Bio-hythane volumetric containing 10–30% hydrogen and 70–90% methane, as an advanced gaseous bio-fuel could be potentially produced by the two-stage anaerobic co-digestion process, which is one of simply practical approaches to simultaneously producing high added-value energy carrier and efficient stabilizing waste. Previously, batch two-stage anaerobic co-digestion of skim latex serum (SLS): *Rhizoclonium sp.* macro-algae at VS basis mixing ratio of 6:4 could provide synergisms, resulting in satisfactory bio-hydrogen and methane potential (BHP and BMP) of 67.2 ± 2.9 mL H_2/g-VS_{added} and 326.5 ± 13.6 mL CH_4/g-VS_{added}, respectively. The two-stage process for continuous hydrogen and methane production was subsequently investigated in the continuous stirred tank reactor (CSTR) and up-flow anaerobic sludge blanket (UASB) reactor, respectively in order to assess the potentiality of using SLS to anaerobically co-digest with *Rhizoclonium* sp. under thermophilic conditions (55 °C). By feeding with initial concentration of 46.3 g-VS/L of mixed substrates, having VS based SLS: *Rhizoclonium* sp. mixing ratio of 6:4, specific hydrogen yield of 63.5 ± 2.35 mL-H_2/g-VS_{added} was obtained by operating CSTR at hydraulic retention time (HRT) of 2 days. Meanwhile, methane yield of 310.4 ± 13 mL-CH_4/g-VS_{added} was obtained by operating UASB reactor at 18-day HRT. Economical hydrogen and methane yields of 2.87 L-H_2/L-substrate and 14.1 L-CH_4/L-substrate obtained simultaneously with organic removal of more than 85% from the continuous two-stage anaerobic process could be enable potentially for scale-up this two-stage process to the industrial scale for further investigation in process performance and stability of co-digestion SLS and *Rhizoclonium* sp. to generating high added-value bio-hythane, effectively.

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P23-25

Bioengineering of yeast cell for biodiesel production

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Due to increased oil demand, depleting fossil fuels and greenhouse gas emissions, biofuels production are getting much attention. The fatty acid based biofuels (fatty acids ethyl ester/biodiesel, fatty alcohol, etc.) produced from microbial cells have emerged as ideal alternatives to fossil oils, with significant pluses over the plant, animal and algae oils. *Saccharomyces cerevisiae* is a most studied industrial model microorganism and also its fatty acid production ability has been increased by metabolic engineering approach. But still, the cost of the process limits its industrial production, therefore, more research is required. Here, we are addressing this issue by sequential metabolic engineering approach.

In order to synthesize biodiesel in yeast cells, we integrated wax ester synthase (WS2) gene from *Marinobacter hydrocarbonoclasticus* into its genome. The genetic engineering approaches have focused on high-level biodiesel production by rewiring metabolism pathways to upsurge carbon flux towards fatty acid CoA synthesis, by increasing the cofactor supply and disrupting the degradation pathway.

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P23-26

Comparative studies for bioethanol production using agricultural and forest wood residue

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Lignocellulosic biomass as a second generation bioethanol is abundantly available, carbon-neutral and can serve as a renewable energy source that can replace fossil-derived fuels. Cellulose, hemicellulose and lignin form the principle components of lignocellulose. Lignocellulosic biomass from forest and agricultural residues is an inexpensive source of polysaccharides. Pretreatment techniques involve delignification of feedstock in order to make cellulose and hemicellulose more accessible to hydrolysis. Pretreatment methodology for lignocellulose based raw materials differs according to physical structure, chemical composition and ratio of constituents of biomass. Woody substrate from forest residue i.e. hardwood and softwood contain different concentrations of cellulose, hemicellulose and lignin. Depending upon the chemical composition of agricultural and forestry residues the thermochemical pretreatment optimized was 1% nitric acid at 10% biomass loading giving maximum reducing sugars i.e. 7.1913 g/L for ethanol production in case of Sesamum stalk. For forestry residue 1% hydrochloric acid at 5% biomass loading gave maximum reducing sugar yield of 7.90793 g/L for Pinus needles, 6.88441 g/L for *Cedrus deodara* sawdust and 2.45621 g/L for *Shorea robusta* sawdust (at 15% loading). The statistical optimization of pretreatment parameters was accomplished by Central Composite Design (CCD) tool of Response Surface Methodology (RSM) using statistical software Design expert 6.0, Stat Ease, Inc., Minneapolis, Minnesota, USA. Enzymatic hydrolysis of pretreated samples was investigated using commercial enzyme CELLULASE ONOZUKA R-10 from Trichoderma viride. Comparative studies using simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) has shown highest bioethanol yield of 1.280% (v/v) using SHF with Pinus needles.

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P23-27

Future perspective of algae: energy production and environmental cleanup

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The research emphasis is on the current progress of algal biofuel production with efficient strain selection and algal cultivation systems, biomass production and technology as well as the possibility of eco-friendly wastewater treatment and greenhouse gas mitigation and bioelectricity generation. The experiments were conducted in an Algal Microbial Fuel Cell (AMFC photobioreactor (single-chamber). The pH, EC (electrical conductivity), COD (chemical oxygen demand) and TDS (total dissolved solids) decreased

from 8.01 to 7.0, 982 to 854 (mS/cm), 255 to 112 (mg/L) and 490–427 (mg/L), respectively, over course of 7 days. Biomass production, rate of biomass production, chlorophyll a, b and "total chlorophyll" content increased with increasing time and were observed to be 3300 mg/L, 471.42 mg/L/day, 0.981 mg/L, 0.173 mg/L and 1.156 mg/L after 7 days. Lipid production and rate of lipid production were 1068.383 mg/g dry wt. biomass and 152.62 mg/g dry biomass/day. FTIR (Fourier transform infrared) spectra revealed the presence of protein, lipid, FAs (fatty acids), triglycerides and ester functional groups. FAME (fatty acid methyl esters) profile revealed the presence of C16:0, C18:2n-6, C18:1 and C16:1. The generation of electric potential by *Leptolyngbya* sp. JPMTW1 increased significantly ($p \leq 0.05$) from 0.0211 to 0.264 mV within 7 days. The maximum power density (0.008 mW/cm^2) was obtained at cell potential at 12 mV. This study shows that simultaneous production of biofuel, bioelectricity and wastewater treatment is possible by *Leptolyngbya* sp. JPMTW1.

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P24-1

Residence time distribution in packed bed microreactor – Lattice Boltzmann model validation

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Microfluidic devices offer numerous advantages for biocatalytic processing such as small size and high surface-to-volume ratio leading to very efficient mass and heat transfer, continuous operation mode and improved safety, among others [1]. Novel microreactor designs, such as packed bed microreactors (mPBRs) aim to harness these advantages and lead to faster process development and intensified production. Furthermore, they offer simplicity regarding assembly and scale-up/-out.

In our work, a micro packed-bed reactor (μPBR) based on two-parallel-plates configuration with immobilized *Candida antarctica* lipase B in the form of porous particles (Novozym® 435) was theoretically and experimentally characterized. A residence time distribution (RTD) within μPBRs comprising various random distributions of particles placed in one layer was computationally predicted by a mesoscopic lattice Boltzmann (LB) method. Numerical simulations were compared with measurements of RTD, obtained by stimulus-response experiment with a pulse input using glucose as a tracer, monitored by an electrochemical glucose oxidase microbiosensor integrated with the reactor. The model was validated by a good agreement between the experimental data and predictions of LB model at different conditions. The developed μPBR was scaled-up in length and width comprising either a single or two layers of Novozym® 435 particles and compared regarding the selected enzyme-catalyzed transesterification.

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P24-2

Microfluidic diffusion analysis of the size distribution and micro-rheological properties of antibody solutions at high concentrations

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The size distribution and rheological properties of dispersions of biological colloids are relevant quality attributes for a variety of industrial applications, including pharmaceutical, food and cosmetic products. For instance, the biophysical properties of monoclonal antibodies and therapeutic proteins, representing an important class of drugs in the pharmaceutical market, are important for their safety and efficacy. In this work, we apply a microfluidic diffusion platform to analyze protein sizes and interactions in high concentration antibody solutions directly in the liquid state with minimal perturbation of the sample. We show that this method provides size distributions in a size range scaling from a few angstroms to hundreds of nanometers. The detection sensitivity of the technique is independent of the particle size and the method provides number-average distributions, enabling the simultaneous detection of both monomeric species and soluble aggregates. We further show that the same platform can be applied to measure viscosity-scaling effects in crowded environments by probing the Brownian motion of several tracers with different sizes. Such tracers experience a shift from the micro-viscosity to the macro-viscosity of the sample at a critical probe size that is equal to the characteristic dimension of the main components of the dispersion. The technique simultaneously provides quantitative measurements both on the micro-rheological properties and the macro-viscosity of the sample and on the characteristic size of the components of the solution. Overall, these methods represent attractive tools in the context of the analysis of sizes and interactions of proteins in both diluted and high concentration solutions during development, manufacturing and formulation.

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P24-3

Bioprocess engineering of stable insect cells for enhanced production of HIV Gag-VLPs for drug and vaccine development

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Conformational-complex membrane proteins (MPs) are vaccine/drug targets in many diseases, but their full implementation has been slowed down by the lack of efficient production tools. Co-expression of MPs with matrix proteins from enveloped viruses is a promising approach to obtain correctly folded proteins at the surface of ordered nanoscale architectures such as virus-like particles (VLPs), preserving their native lipidic environment.

In our lab, we have been developing reusable, stable insect cell lines for production of VLPs pseudo-typed with target MPs [1], but the titers achieved to date are still seemingly low.

Aiming to increase productivities, bioprocess engineering schemes were implemented (either separate or combined): (i) adaptive laboratory evolution (ALE) of cells to hypothermic culture conditions, and (ii) supplementation of cell cultures with productivity enhancers. Maximum HIV Gag-VLPs expression was obtained

using ALE alone, with up to 30-fold increase in titers when compared to control cultures (parental cells cultured at standard 27 °C).

The suitability of the newly developed adapted cell line for pseudo-typed VLPs production was verified with the generation of Gag-HA VLPs (vaccine candidate). When compared to control cultures, these cells showed increased Gag-HA VLPs expression thus corroborating previously obtained data. Bioprocess intensification strategies (e.g. perfusion) are currently under in-house testing to further improve yields.

Overall, the insect cell platform and bioprocess engineering strategies herein assembled have the potential to assist and accelerate drug and vaccine development.

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P24-4

Comparison of ionic liquids for efficient pretreatment of brewery spent grain

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During the elaboration of beer, highly useful derivatives are also obtained, including brewery-spent grain (BSG). Their cell walls could be degraded by hydrolystic processes to release sugars that could be further employed as precursor of other add-value compounds or enzymes by microbial transformation. Green Chemistry principles have urged the scientific community to invest more research efforts in the design of more sustainable strategies. This work bets in the application of aqueous solutions of a cholinium-based ionic liquid (IL) containing glycinate as anion [N_{1112OH}][Gly] and a conventional imidazolum-based IL (1-ethy-3-methylimidazolium acetate, [C_2C_1im][C_1COO]) under the same operating conditions (90 °C). Although the recovery of carbohydrate-rich material (CRM) is slightly higher (43.3%) with [C_2C_1im][C_1COO] vs 32.0% with [N_{1112OH}][Gly], the values of total lignin reduction are sweepingly lower, since Klason lignin was reduced by just 11.23%, being the total reduction of lignin of only 40.18%. In the same line, the saccarification yields were also improved from 64.85% with raw BSG and 62.13% with [C_2C_1im][C_1COO] up to 94.26% with [N_{1112OH}][Gly]. The results presented in this work make up the basis for a rational design of bio-ILs for delignification of lignocellulosic materials.

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P24-5

Co-production of menaquinone-7 and nattokinase by *Bacillus subtilis* with a dissolved oxygen control strategy

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To produce menaquinone-7 (MK-7) and nattokinase (NK) using soybean curd residue, *Bacillus subtilis* was employed along with medium optimization and dissolved oxygen (DO) control. We found that the concentration of MK-7 and NK exhibits a positive correlation with different *Bacillus subtilis* strains. Subsequently, response surface methodology was employed to optimize the medium used for the simultaneous production of MK-7 and NK. The optimized medium contained (%, w/v) 12.2% soybean curd residue, 5.7% soya peptone, 2.6% lactose and 0.6% K_2HPO_4, resulting in MK-7 levels of 44.72 mg/L and NK levels of 2118.26 U/ml. The DO plays an important role in the production of MK-7 and NK. With the increase in DO concentration, cell growth and NK activity were improved. In the low DO concentration conditions, MK-7 production rapidly increased in the late fermentation steps. A novel co-production strategy for MK-7 and NK was developed by controlling aeration rates in the process of fermentation. The concentrations of MK-7 and NK achieved were 91.25 mg/L and 2675.73 U/ml, respectively.

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P24-6

An inertial microfluidic device for targeted cell separation

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In this paper, we present an inertial microfluidic device with a symmetric serpentine channel to separate MCF7 cancer cells form other cell types with a high efficiency. Manipulation of particles and cells has various applications in clinical operations and industry. In all of the separation methods, a label-free technology without the use of filter, antibody affinity or centrifugation is highly favorable to lower damage and changes in the cells situation. Inertial microfluidics is one of the most suitable label-free technologies with a high throughput. It is common to use a curved channel in this approach to achieve separation of particle and cells through balancing of the inertial lift force and the secondary flow drag force. The low aspect ratio of the channel cross section prevents the mixing effect of the secondary flow. The separation concept is based on the fact that cells with different sizes are focused on different equilibrium positions of the particles perpendicular to the flow. To calculate inertial lift force, which is exerted on the particles from the fluid, and the effect of particles motion on fluid flow the interaction between the fluid and particle is investigated accurately through implementation of 3D Direct Numerical Solution (DNS) method. The results indicate that this device has some advantages over other existing microfluidic separation techniques, including better purity, efficiency, footprint, parallelizability throughput and resolution. The results of this study can be applicable for facilitated target cell separation with high efficiency in the clinical applications.

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P24-7

Enhanced oxygen mass transfer in an airlift bioreactor with helices

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Efficient oxygen mass transfer is often the bottleneck factor in aerobic bioprocesses. Thus, it is necessary to know the critical factors affecting it. A draft tube sparged airlift bioreactor (V = 116 L) was occupied with helical flow promoters in the downcomer and the riser zones of the bioreactor. Besides working as flow promoters, the helices were used for extra aeration either separately or at the same time. The capacity of the airlift bioreactor with helical flow promoters to transfer oxygen to the system was evaluated by a volumetric oxygen mass transfer coefficient (k_La) with water and non-Newtonian solution.

The airlift bioreactor with helices had significantly lower power inputs compared with traditional stirred tank bioreactors with similar oxygen transfer coefficients when water was used as the working medium. The power requirement of airlift bioreactor with helices for a level of k_La 0.1 s^{-1} was less than 20% of the power requirement of a typical stirred tank bioreactor for the same level of k_La. In non-Newtonian medium, it was observed that aeration through the helix in the downcomer zone could improve the k_La level in the bottom zone of the airlift bioreactor significantly, even 60% with only 7% increase in energy input compared with a conventional draft tube airlift bioreactor. This is very promising as the bottom zone is typically the weakest part of bioreactors when speaking about oxygen mass transfer.

The airlift bioreactor with helical flow promoters seems like a promising energy efficient bioreactor type for processes with high oxygen demand.

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P24-8

Effect of oxygen transfer on microbial SAM production

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S-Adenosyl methionine (SAM, also known as AdoMet and SAMe), exists in all living organisms, and serves as an activated group donor in a range of metabolic reactions including: methylations, trans-sulfuration and propylamine group donations. It can protect the liver against lesion agents and has been administered as human therapy for depression syndromes and osteoarthritis with excellent results. Microbial SAM, with diverse pharmaceutical applications, is biosynthesized from L-methionine-enriched media under aerobic condition by using a mutated yeast in this study. To compare the SAM productivity under different oxygen transfer modes, dissolved oxygen (DO) control in the fermenter was carried out. The results show that the conversion rate of L-methionine and cellular SAM content are higher with the help of high oxygen transfer provided by increased impeller speed. Compared to the fermentation in the shaking flask, of 100 ml with 200 rotation speed, an additional increase of the cellular SAM concentration in the fermentation reaches 19.4% and the overall SAM amount increases 33.5% under the condition that the DO was controlled above the 10% of saturation.

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P24-9

Application of the enzymatic fuel cell system using waste microalgal hydrolysate

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Microalgae have recently been considered as the most promising renewable feedstock for biorefinery. The purpose of this study is to generate power by using the waste hydrolysate from microalgal extracts (AH), as substrates of enzymatic fuel cell (EFC) containing sugars, and analyze the overall effects of the microalgal extract on EFC. In order to extract sugars from microalgae (*Chlorella pyrenoiudosa*), optimum conditions of acid hydrolysis (acid type, solid-liquid ratio) will be established. To obtain high concentration of sugars from microalgae, the reaction conditions for acid hydrolysis were optimized as 100 g/L of solid–liquid ratio, 2% of hydrochloric acid and heated at 121 °C for 15 min. The maximum efficiency 87.56% of hydrolysis was achieved. In order to investigate the effects of various neutralization agents used for AH on the EFC system, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were investigated. Higher current and lower resistance values appeared during the neutralization using KOH. Maximum power density of 3637 W/cm^2 was obtained for the EFC system, 6.59 folds higher than the control. The AH and neutralizing agent have a significant impact on the development of the EFC system and could be utilized as a fuel for power generation in the near future.

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P24-10

High-density Vero cell perfusion culture in BioBLU 5p single-use vessels

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Vero cells are anchorage-dependent cells that are widely used as a platform for viral vaccine production. In stirred-tank bioreactors, they are ordinarily grown on microcarriers. Fibra-Cel disks are a promising alternative attachment matrix with a high surface-to-volume ratio. They provide a three-dimensional environment that protects cells from damaging shear forces, helping to achieve high cell densities. In this study, we cultivated Vero cells in Eppendorf BioBLU 5p Single-Use Vessels pre-packed with Fibra-Cel. The process was controlled with a BioFlo 320 bioprocess control station. We cultivated the cells in perfusion mode, which ensures a consistent supply of nutrients and the removal of toxic byproducts. We achieved the very high Vero cell density of approximately 43 million cells per mL, demonstrating great potential for Vero-cell-based vaccine production using Fibra-Cel packed-bed vessels.

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P24-11

Kinetic characterization of enzymes in a multi-enzyme cascade reaction for the synthesis of statin side-chain precursors

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Statins are a class of medicine that lower the level of low-density lipoprotein (LDL) cholesterol in blood. This can reduce the risk of cardiovascular disease and prevent heart attack and stroke. The modern requirements for stereochemical purity have created new challenges for industrial preparation of statins. One of the ways to achieve the required enantio- and diastereopurities is the use of biocatalysis [1].

In this work, a cascade reaction with three enzymes was investigated for the synthesis of statin side-chain precursors. The first enzyme in this cascade is alcohol dehydrogenase used for the oxidation of an alcohol to aldehyde. The second enzyme is NADH oxidase used for coenzyme regeneration and the third one is deoxyribose-phosphate aldolase for the aldol addition of acetaldehyde and the corresponding aldehyde. All three enzymes were kinetically characterized in order to develop a mathematical model.

Mathematical modeling plays an important part in developing processes involving biocatalysts. In order to reach industrial application, each enzyme and reaction system have to be kinetically characterized. Modelling increases our knowledge about the process, since it enables simulation of different reaction courses. This offers better understanding of the effect that different variables have on the equilibrium, conversion etc. [2].

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P24-12

Bioactive galactoglucomannans extracted from softwood

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Galactoglucomannans (GGMs) being components of wood hemicelluloses play important role in many industries such as pulp and paper industry, textile industry, pharmaceutical industry, food industry and in agriculture. The goal of this study was to examine the possibility of using the substances derived from softwood hemicelluloses as valuable biomaterials.

Small wood chips obtained by mechanical grinding of spruce (*Picea abies*) and larch (*Larix decidua*) wood were used as a raw material. Two main processing methods were used to extract GGMs: thermal method a well as thermal/enzymatic method involving selected enzymes from groups of cellulases and hemicellulases. GGMs precipitate was obtained by adding ethanol to the hemicelluloses solutions obtained by either of above mentioned treatment pathways. Chromatographic techniques such as GC/MS and SEC as well as 13C NMR analysis were used to determine qualitative and quantitative composition of extracted GGMs. The obtained galactoglucomannans were characterized by different chemical composition in terms of glucose, galactose and mannose ratios, and by different average molecular weight, depending on the method of isolation.

A study was also conducted to evaluate the protective and growth stimulating properties of the isolated GGMs with respect to selected plants. The test compositions were prepared on the base of chitosan and softwood extracted GGMs.

The effect of the GGMs addition to the certain fibrous cellulose products on their barrier properties towards air and water vapour was also studied. The fibrous cellulose products containing GGMs showed decreased permeation ability for various gases including air and water vapour.

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P24-13

Development of an efficient biocatalyst system for GABA production

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γ-Aminobutyric acid (GABA) is a four-carbon non-protein amino acid widely distributed in various organism. It has been applied to food and pharmaceutical industries as bioactive supplements. In fact, γ-Aminobutyric acid could provide several physiological functions, inhibition of neurotransmission, induction of hypotensive, treatment of epilepsy and tranquilizer effects, vital to human health. Glutamate decarboxylase (GAD) is capable to catalyze the conversion of glutamic acid to γ-aminobutyric acid. As known, GABA producing microorganisms are usually lactic acid bacteria, capable to produceγ-aminobutyric acid extracellularly. However, due to complicated compositions of the fermentation broth, large scale-purification of GABA seemed to be not cost-effective. The enzyme transformation of GABA is proposed herein as a promising method due to simple reaction procedure, high catalytic efficiency, mild reactions and environmental compatibility. The main aim of this work was to develop an efficient process for industrial production of GABA by biocatalyst system. In this study, glutamate decarboxylase constructs pBAD-GAD was expressed in *Escherichia coli*. The apparent molecular mass of GAD was estimated as 52 kDa by the SDS-PAGE analysis. To improve production yield of glutamate decarboxylase with recombinant *E. coli*, the concentration of inducer (L-arabinose), glutamic acid, glucose, PLP, the induction time, medium compositions and culture conditions were investigated. The results shows that *E. coli* DH5α/pBAD33-gadA using M9 medium (0.3% disodium hydrogen phosphate and 0.5% yeast extract) with 0.5% glutamic acid, 1% glucose, 10 mM arabionse, 0.02 mM PLP and 40 °C and pH 6.5 for 22 h could obtain 1.26 g/L GABA production.

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P24-14

Enhanced high-molecular-weight exopolysaccharides production via *Tuber borchii* cultivation

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Truffle with its special aroma and taste in food market has a higher economic value in the edible mushrooms market. In this study, *Tuber borchii* was cultivated in the submerged culture to produce *Tuber mycelial* biomass and its exopolysaccharides (EPS). It was found that the pH, carbon and nitrogen sources in the medium gave a significant influence on the mycelial growth and metabolites production. Results showed that an initial pH of 7 and a rotation rate of 100 rpm are the best conditions for Tuber to produce EPS (244 mg/L). As to the carbon sources, sucrose was found to be the best for Tuber EPS production. When 80 g/L sucrose was used, an EPS yield of 548 mg/L could be reached. For the nitrogen sources, yeast extract was found to be the most favorable source for Tuber EPS production. When using 20 g/L of yeast extract, an EPS of 725 mg/L was observed. In addition, the high-molecular-weight EPS occupied the highest ratio (45%) of all the EPS when 20 g/L yeast extract was used in the medium. In the time course, the biomass and EPS were found to give the highest yield at day 28, where a highest molecular weight EPS of 208 kDa was also obtained.

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P24-15

Production of secondary metabolites by *Aspergillus terreus* cocultivated with *Chaetomium globosum* under submerged conditions

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The present communication describes the results of shake flask cocultivation of *Aspergillus terreus* ATCC 20542 and *Chaetomium globosum* ATCC 6205. The onset of cocultivation was marked by transferring the spores of both strains from agar slants into sterile liquid medium containing lactose and yeast extract as the sources of carbon and nitrogen, respectively. In the case of two-stage fermentation the 24-h preculture was used to inoculate the production medium. In one-stage fermentation the preculture was not included and the spores of both strains were transferred directly from agar slants into the production medium. The monocultures served as experimental controls. After 7 days of cocultivation the levels of secondary metabolites produced by *A. terreus* were determined with the use of liquid chromatography coupled with high-resolution mass spectrometry. In addition, the chromatograms corresponding to cocultures and monocultures were compared in the context of the displayed biosynthetic repertoire.

Irrespective of the cultivation strategy, the concentration of lovastatin in cocultures was lower than in the *A. terreus* monocultures. The opposite observation was made with regard to butyrolactone I levels. In contrast, the biosynthesis of (+)-geodin and asterric acid was either enhanced or inhibited by the presence of *C. globosum* depending on whether one-stage or two-stage fermentation was performed. The differences observed between the two tested strains with respect to spore size and shape opened the door for microscopic observations of morphological events occurring during the first 24 h of cocultivation. Interestingly, the experiment revealed that the spores of *A. terreus* and *C. globosum* tend to coagglomerate in submerged cultures.

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P24-16

Magnetic biocomposites as support for trypsin immobilization: Application for protein digestion

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Magnetic nanoparticles (MNPs) show many valuable features, including good thermal stability, biocompatibility or chemical inertness. MNPs are also used as a precursor and combine with other materials to produce functional hybrids, which might be applied in many various branches of industry. However, in recent years, magnetic hybrid materials, have been intensively studied as an enzyme carrier, due to their ferromagnetic properties, which facilitate simple and efficient separation of magnetic-based biocatalytic systems form reaction mixture using an external magnetic field.

Thus in presented work magnetic nanoparticles were combined with biopolymers – chitin and lignin – to prepare novel magnetite-chitin and magnetite-lignin hybrid materials. The synthesized supports were thoroughly analyzed and further used for immobilization of trypsin. During the research quantity of immobilized enzyme and immobilization yield were evaluated as well as kinetic parameters of free and immobilized trypsin were examined and compared. Finally, produced systems were applied as biocatalytic tools for human serum albumin digestion.

Effective enzyme immobilization was confirmed and high immobilization yields were achieved. Storage stability and reusability of the immobilized trypsin was significantly enhanced, compared to free enzyme. Results obtained after protein digestion confirmed that produced biocatalytic systems exhibited high proteolytic activity. Moreover, it should be emphasized that presented in this study results clearly shows that synthesized magnetic hybrid materials might also be applied for immobilization of enzymes from various catalytic classes.

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P24-17

Mathematical model based optimization of enzymatic aldol addition of propanal to formaldehyde

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Carbon–carbon bond formation between propanal and formaldehyde in aldol addition catalysed by newly developed D-fructose-6-phosphate aldolase variant was investigated in this

work. The reaction product is 3-hydroxy-2-methylpropanal (Roche aldehyde) widely used as intermediate in synthetic chemistry. The reaction kinetics was determined and the mathematical model was developed for process optimization. Optimization goals included finding the best conditions and the best reactor mode to obtain maximum product concentration and yield. It was found that the kinetics of the aldol addition can be described by double substrate Michaelis–Menten kinetics with included substrate inhibition by formaldehyde and propanal and noncompetitive inhibition by methanol used as formaldehyde stabilizer. Self-addition of propanal was described by double substrate Michaelis–Menten kinetics and included in the model. It was found that enzyme operational stability decay is co-dependent on the initial formaldehyde concentration. Thus, the choice of the initial conditions is crucial for the successful process set-up. Developed mathematical model described the experimental data very well. At the calculated optimal process conditions in fed-batch as the best reactor choice, the product concentration after 5.5 h was 814 mmol dm^{-3} (72 g L^{-1}), product yield was 88.5% and volume productivity was 313.7 g L^{-1} d^{-1}.

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P24-18

Dual colorimetric and ratiometric fluorescent pH sensors for neutral and alkaline pH ranges

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In this study, coumarin 6 (C6) or nile blue A (NB) was captured into melamine-formaldehyde (MF) resin particle. The dye-doped MF particles were incorporated with the polymer Nafion or polyurethane hydrogel for the fabrication of the pH-sensing membranes based on the change in color and fluorescence spectra. The shift in fluorescence emission wavelength of the MF-C6 membrane at neutral and acidic solutions was used as a ratiometric fluorescence modality for the linear range of pH 4.5–pH 7.5, whereas that of the MF-NB membrane was based on the change in fluorescence intensity of two band edges of NB in alkaline solutions to get the linear range of pH 9–pH 12. Both the MF-C6 membrane and the MF-NB membrane exhibited color transition under normal visual sense, i.e., from yellow to pink color of the MF-C6 membrane at neutral and acidic solutions and from blue to purple color of the MF-NB membrane upon exposure to solutions of pH > 10. These pH-sensing membranes also showed high sensitivity, good selectivity and reversibility, and also long stability, which could be often used in a few specific applications.

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P24-19

Chemical engineering methodology in process development: A case study of MenD-catalyzed 1,4-addition of α-ketoglutaric acid to acrylonitrile

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Kinetics of the MenD-catalyzed 1,4-addition of α-ketoglutaric acid to acrylonitrile were investigated in detail and based on the results a mathematical model of the process was developed and experimentally validated. The reaction kinetics were described by the double substrate Michaelis–Menten kinetics where the reaction rate linearly depends on acrylonitrile concentration. Non-competitive enzyme inhibition by p-methoxyphenol, i.e. acrylonitrile stabilizer, was also included in the model. Spontaneous reactivity of acrylonitrile towards polymerization was noted. Based on the experimental results, as well as model simulations, a fed-batch reactor was selected as the best reactor mode to carry out the reaction due to shortened reaction time compared to the repetitive batch reactor mode, and the ability to obtain maximum yield on the product, i.e. 6-cyano-4-oxohexanoic acid. Additionally, the fed-batch reactor method enables to set up the feeding strategy of acrylonitrile necessary to keep its concentrations below the level which significantly affects enzyme operational stability. The mathematical model was used for process optimization and as an assessment tool for the economic evaluation. Results have shown that in the investigated variable range all the requirements for the industrial implementation can be achieved; i.e. product concentration of 70 g L^{-1}, volume productivity of 58 g L^{-1} d^{-1}, product yield of 96%, and biocatalyst yield of 28.9 gP $gMenD^{-1}$. Based on the optimized production procedure, economic analysis was performed to determine the breakeven point of the process. In addition, influence of uncertainties in estimation of input variables on overall economic performance was assessed using sensitivity analysis.

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P24-20

Evaluation of γ-aminobutyric acid (GABA) production from waste feedstocks by an isolated *Bacillus subtilis*

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The γ-aminobutyric acid (GABA) is a functional amino acid and draw much attention for researchers due to its medical potential. The objective of this study was to evaluate an optimized culture medium comprised of waste feedstocks and maximize the productivity of GABA. First, *Bacillus subtilis* BBEL02 was screened from fermented kimchi. Soybean hydrolysate and corn steep liquor were employed as nitrogen supplement for GABA production, and the effects of different carbon sources were also investigated. The results indicated that a modified defined medium with 1% of glucose and 2% of soybean hydrolysate can reach highest GABA concentration at 1.623 g L^{-1}, and GABA productivity of 541.2 mg L^{-1} day^{-1}. It showed that the SBH characterized with

higher glutamic acid content could be a potential feedstock for GABA production.

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P24-21

Kinetic model describing the early stages of morphological evolution of various filamentous fungi species

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Filamentous fungi produce enzymes and metabolites, which are applied in medicine or food technology. They exert various mechanisms of pellets formation in the submerged cultures.

This work is aimed at the formulation of the kinetic model to describe the early stages of mycelial evolution of various fungal species: spore aggregating *Aspergillus terreus*, hyphae aggregating *Penicillium rubens* and *Chaetomium globosum* and non-aggregating *Mucor racemosus* in the standard and microparticle-enhanced cultivations (MPEC). The experimental data for the estimation of model parameters were derived from digital analysis of the microscopic images of mycelia cultivated in shake flasks and bioreactor.

Mean projected area (A, μm^2) of hyphal objects (spores, agglomerates or hyphae) was the dependent variable in the model. Three phases of morphological evolution of fungal species were considered and their rates were: spore swelling (r_{SW}):

$$r_{SW} = k_{SW} \times A$$

where k_{sw} is swelling rate constant (h^{-1})–spore or hyphae agglomeration (r_{AGL}):

$$r_{AGL} = k_{AGL} \times A \times \exp\left(-\frac{A}{k_{AGL}}\right)$$

where k_{AGL} is agglomeration rate constant (h^{-1}) and K_{AGL} limitation constant due to the size of agglomerate (μm^2)–mycelial growth (r_G): where k_G is growth rate constant (h^{-1}) and K_G limitation constant due to the size of agglomerate (μm^2).

The increase of the size of the mycelial objects was limited by themselves. Neither limitations from media components nor oxygen saturation occurred. The balance equation was as follows:

$$\frac{dA}{dt} = \begin{cases} k_{SW} & \text{if } t < 5\,h \\ \max(r_{AGL}, r_G) & \end{cases}$$

for spore agglomerating species, or

$$\frac{dA}{dt} = \begin{cases} k_{SW} & \text{if } t < 5\,h \\ r_{AGL} + r_G & \text{otherwise} \end{cases}$$

for other species.

Large differences between the values of kinetic parameters for the studied fungi were found. For example, agglomeration rate constant $\underline{k}_A$ of *Aspergillus terreus* was equal to 1.79 h^{-1} (standard cultivation) and 1.34 h^{-1} (MPEC), while *Penicillium rubens* reached 3.99 h^{-1} (standard cultivation) and 1.83 h^{-1} (MPEC).

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P24-22

Effect of different modifying oxides addition on the cytocompatibility of phosphate based glasses

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For the last few decades, there has been a growing interest in using glasses for biomedical applications. Phosphate based glasses (PBGs) are known to show good bioactive characteristics and could be potentially used as favourable templates for bone-tissue formation. Phosphate glasses are a unique group of materials that offer great potential for hard and soft tissue engineering over other types of bioactive glasses due to their fully resorbable characteristics, with some formulations possessing chemical composition similar to the mineral phase of natural bone. The biocompatibility of these glasses are hugely affected by the glass composition which could be easily altered via the addition of different modifying oxide. The main aim of this current work was to establish a relationship between the ion release and cytocompatibility of different formulations of PBGs. Different modified oxides (Fe2O3, B2O3, SrO, Al2O3) were added to the glasses in order to observe the effect of composition on the durability of the glasses. Ion release studies were conducted using Inductively Coupled Plasma Spectroscopic method. In order to observe the relationship between the ion release, degradation rate and cytocompatibility of the glasses cell culture studies were conducted using human osteoblast like (MG63) cell lines. It was revealed that the glasses with higher durability showed higher cell viability. Different ions released from the glasses also showed a significant effect on the cytocompatibility. Therefore, it was concluded that a careful selection of the glass composition is essential to achieve better cytocompatibility properties to be used in different biomedical applications.

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P24-23

Integration and expression of the polyhydroxybutyrate biosynthesis-related gene in *Bacillus subtilis*

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Polyhydroxyalkanoates (PHAs) belong to polyesters that attracted attention in the field of biodegradable plastics. For environmental stress, bacteria could synthesize PHAs as carbon storage in nature. Polyhydroxybutyrate (PHB) is the most ordinary type of PHAs. *Bacillus subtilis* can be used as a safe producing strain for PHB synthesis because of it is generally recognized as a safe (GRAS) strain and is widely utilized for the production of commercial proteins and chemicals. In previous work, we introduced PHB synthesis genes from *Ralstonia eutropha* and *Bacillus megaterium* into *B. subtilis* for PHB production. To achieve the purpose of stable production, the PHB expression cassettes were integrated into *B. subtilis* chromosome. Results showed that the new recombinant *B. subtilis* bearing these PHB expression cassettes could stable production the PHB without the addition of antibiotics. In the future, the metabolism and expression will be regulated to increase the PHB production.

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P24-24

Crude cellulase treatment for reactivity enhancement of pre-hydrolysed kraft dissolving pulp for viscose

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The application of cellulase enzymes to enhance the pulp reactivity and accessibility for viscose is an active research area. Dissolving grade pulp consists of almost 90–98% of cellulose with a low content of hemicelluloses, minerals, extractives, and residual lignin. Cellulases have been widely used to improve the pulp reactivity due to their etching action. To improve the process economics of reactivity improvement, crude cellulase from *Bacillus subtilis* was employed for the treatment and significant dissolving pulp properties were analyzed. With increase in enzyme dose from 0.25 U/g to 2 U/g, improvement in Fock reactivity and alkali solubilities (S10 and S18) was observed with simultaneous reduction in viscosity and yield. Fourier-transform infrared spectroscopy and scanning electron microscopy were used to observe the molecular level effects on dissolving grade pulp.

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P24-25

Development of online monitoring in simple cultivation vessels

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Although novel parallel cultivation systems were developed in the last couple of years, shake flasks are still the workhorse of microbial cultivation. Typically, these vessels are still used as black boxes because no online measurement is integrated. The non-invasive measurement of oxygen and pH using chemical-optical sensors has already been commercially available for several years and online measurement of biomass has recently been introduced. Here, we present the new CO_2 sensor that was developed recently and integrated into a multi-parameter platform.

Applications are various: Although the CO_2 sensor is only a prototype it is possible to follow a diauxie of *E. coli* cultivations online, while small changes in the growth curve detected by the biomass sensor indicate the exact time of limitations which was shown for different organisms. Non-invasive oxygen measurements deliver the critical process parameter kLa–which is even the basis of online OUR determination.

However, our focus was not just to add more parameters but also to let them complement each other. Measur-ing several parameters – with all of them showing the same characteristics – enhances the measurement security. It was shown that combined oxygen and biomass measurement offers a solid conclusion about the metabolic status of the culture. In summary, multisensory monitoring enables to adjust the conditions in shake flask cultivations to be far more comparable to stirred bioreactors. Therefore, scale-up with yield optimization can be performed more reproducibly.

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P25-1

Assessment of potentially functional hydrocarbon-degrader bacterial communities in response to *Micrococcus luteus* EOM using culture-dependent and culture-independent methods

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The efficiency of bioremediation using indigenous microbial community to attenuate the contaminations depends on the survival and metabolic activities of the microbes involved. From the natural microbial microflora, only few species can be cultivated by classic culture-dependent methods: most of the microorganisms remain uncovered. The unculturable strains are frequently in a viable but not culturable (VBNC) state which might be converted into culturable or metabolically activated status by resuscitation promoting factors (Rpfs) such as extracellular organic matter (EOM) producing by *Micrococcus luteus*.

Lubricating oils (LOs) of varying composition are mostly produced for reducing friction in engines of motorized vehicles, such as cars, motorcycles or locomotives. These contain long chain hydrocarbons, additives and – after usage – heavy metals, which are considered as widespread, hazardous pollutants and hence potential targets of different rehabilitation processes. Monitoring an oil contaminated site, a natural microflora was characterized by metagenomic tools. The indigenous microflora was adapted to higher used lubricating oil (ULO) concentrations and changes in microbial communities were followed.

Since the detailed analytics of ULOs is difficult, artificial hydrocarbon mixtures were used to test the performance of the ULO-adapted cultures. Biostimulation, bioaugmentation experiments together with the usage of EOM of *M. luteus* were performed and the bioconversion efficacies of the various hydrocarbons were monitored.

Our results clearly indicate the applicability of the EOM of *M. luteus* for promoting the bioremediation processes and demonstrate selective effect of EOM on the bioconversion yields.

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P25-2

Withdrawn

P25-3

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P25-4

Isolation and identification of PAH degrading bacteria from Jamaican Soil, Landfill Leachate, Kingston Harbour: Water and sediment samples

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16s rRNA gene sequence homologies in both the Ribosomal Database Project and the NCBI GenBank, bacteria of the genera *Escherichia* and *Shigella* have been isolated using a regime to select for PAH-degraders in Jamaica.

PAH-degrading bacteria were isolated from various sites on the shores of the Kingston Harbour and land sites deemed to have been exposed for at least 15 years of anthropogenic PAH contamination around Kingston, and one rural automotive repair yard (in a typically "oily and plastic-burning garbage-burning" category).

16S rRNA gene sequences of isolated PAH-degrading bacteria were compared to libraries. None of the isolates showed a 100% similarity with the 16S rRNA gene sequences in the GenBank database nor with the Ribosomal Database Project: this could imply that these isolates are novel strains.

The 16S rRNA gene sequences of the CM1 and CM7 isolates showed a 99% homology to bacteria of the genera *Paracoccus* and *Aeromonas* respectively, CM5 showed 99% homology to bacteria of the genera *Enterobacter*, *Pantoea*, *Leclercia* and *Klebsiella*, CM6 showed 99% homology to *Stenotrophomonas* and *Pseudomonas*, while CM4 showed 99% homology to the genera *Escherichia* and *Shigella*.

Keywords: PAH degradation, Escherichia, Shigella, Jamaica, bioremediation.

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P25-5

Harvesting from the extreme and polluted: Extremophile microalgae living and recovering Uranium

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In the current scenario of anthropogenic global change, the study of newly evolved extremotolerants engendered by human-induced extreme environments gains new insights. A dramatic example of human-generated harsh environments is the uranium (U) mining and milling areas. U mine tailings pollute extensive areas worldwide which impact in situ and *ex-situ* prevail during long time scales even after the cessation of activities. Noteworthy, microbial species able to adapt to U contamination successfully are relevant for applied bioprospection. An illustrative example of these mine environments can be found at U mine in Saelices (Salamanca, Spain) exploited since the 1970s and currently under restoration. From this location, we have studied isolated and identified a strain microalgae, *Chlamydomonas* sp. (ChlSP strain), that inhabits extremely U mine evaporation ponds, contaminated with U and other metals as well as acidic and radioactive. The ChlSP strain characterization subjected to BLAST analyses resulted in very low values (<92%). Afterward, we have subjected to selection the ancestral ChlSP strain, increasing the U uptake capacity by 30%. Moreover, the kinetics of U uptake bioassay indicated that the selected strain is able to remove close to 4 mg U L-1 in 24 days. Anthropogenic pollution extremophiles have the unique ability of "living on the edges" of contamination, being target microorganisms for the study of adaptation and a source of biotechnological and bioremediation novelty.

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P25-6

Lipase production from *Cryptococcus albidus* D24 using disposed automotive oil

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Automotive oil is one of the products obtained from petroleum distillation. After being discarded from automobiles, it is then classified as a disposed automotive oil, which is mainly composed of long chain saturated aromatic hydrocarbon. Various studies had confirmed that disposed oils constitute potential threats to the environment and public health. The harmful effects of disposed oil can be annihilated through the biodegradation by microorganisms and meanwhile, this oil waste can be used for the production of value-added products such as industrial enzymes.

Lipases, one of the most important groups of industrial enzymes, have a wide range of applications including biofuel and pharmaceutical industries. At this point, isolation and screening of new lipase-producing microorganisms are very important to find lipolytic enzymes having novel properties. Recently, *Cryptococcus albidus* D24 isolated from petroleum sludge have been reported as lipase producing yeast. Since *C. albidus* D24 is isolated from petroleum sludge, it was evaluated as a promising lipase producer using disposed automotive oil. The basal medium used in this work was obtained via central composite design from our previous study. To analyze the usage of disposed automobile oil as raw material, olive oil in the basal medium includes inorganic nitrogen sources and tryptone, was replaced with disposed oil for comparison in terms of lipase production.

The results from this work indicated that disposed automotive oil is a promising raw material for lipase production and the simultaneous biodegradation of a harmful waste oil during lipase production is possible for the benefit of the environment.

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P25-7

Experiments on biofortification of crops by using protein additives based on collagen

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The need for a biobased economy and replacement of oil origin chemicals encouraged the research on development of new additives with biostimulant and nutritional functions from leather industry or aquaculture byproducts. Collagen based byproducts were processed in view of protein separation, and refined by using water extraction, chemical-enzymatic hydrolysis and different steps for filtration, concentration or drying. The physical-chemical characteristics of new additives showed the high versatility of technologies for collagen byproduct processing and tailoring of products with different molecular weights from polypeptides, oligopeptides to free aminoacids. The possibilities to design different materials by collagen additives crosslinking with natural vegetable extracts or encapsulation of essential oils in gelatin shell were experimented in view of new foliar fertilizers development with film forming, slow nitrogen releasing or fungicide properties.

The high potential of collagen to stimulate plant quality and production was investigated on cereal and rape seeds and on different crops, from wheat, rape to leguminous. Laboratory tests allowed to set the toxicological limits and biostimulant properties on germination of cereal and rape seeds. The experiments on different fields showed higher resistance of plants to extreme pH conditions of soil and climate change attributed to biostimulant effect of collagen based additives as an easily available and biocompatible nutrient.

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P25-8

Biodegradation of PCBs from water in *Pleurotus ostreatus* spent substrate-filled bioreactor

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Polychlorinated biphenyls (PCBs) are anthropogenic compounds which belong to persistent organic pollutants. Because of their slow decomposition in nature they still remain an environmental burden despite their ban in the 1980s, and there is a need for a cost-effective and environmentally friendly approach to PCB removal from contaminated sites. In bioremediation the group of white-rot fungi and especially *Pleurotus ostreatus* (the oyster mushroom), was found to have a high degradation potential towards PCBs and PCB metabolites, such as hydroxylated PCBs and chlorobenzoic acids. Moreover, the so-called spent substrate is produced world-wide as waste in commercial mushroom growing farms and can be used for bioremediation purposes. In this work the degradation of PCBs by *P. ostreatus* from water matrix was studied. Firstly, the degradation was tested in flasks in batch setup with *P. ostreatus* inoculated on wheat straw pellets where it was able to remove about 40% of PCBs during 4 weeks of degradation. On the basis of the results a laboratory scale tubular reactor filled with *P. ostreatus* spent substrate was designed. Continuous flow setup resulted in 97% removal of PCBs from artificially contaminated tap water (initial PCB concentration 50 $\mu g\,L^{-1}$; 10 L in total). Finally, the reactor was scaled up (working volume 500 L) and used in trickle bed setup to clean up real contaminated water originated from a site of a former asphalt concrete producing plant in the Czech Republic.

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P25-9

Biodegradation of 2,4,5-trichlorobiphenyl (PCB29) by marine-derived mesophotic fungi

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Persistent organic pollutants (POPs) are organic compounds of natural or anthropogenic origin that resist photolytic, chemical, and biological degradation. POPs bioaccumulate mainly in the fatty tissue of living organisms and have been associated with many health disorders such as cancer, damage to the central and peripheral nervous systems and disruption of the immune system. Although many countries have banned or severely restricted the production and use of POPs in recent decades, these substances are pervasive and can be found in remote environments all around the world.

Marine environments constitute a great pool of diversified natural resources; marine-derived microorganisms and enzymes may possess appealing properties, acquired through millions of years of evolution in unfriendly habitats, which render them valuable for biotechnological applications. The mesophotic zone is an underexplored marine habitat, probably due to the fact that it is below depths (30–100 m) reached with traditional SCUBA diving techniques. The biodiversity of mesophotic coral systems is considered a potential source of novel symbiotic microorganisms, which can contribute to the biodegradation of recalcitrant pollutants such as POPs.

In the present work, we study the ability of 107 microbial symbionts (filamentous fungi) isolated from marine invertebrates, to bioconvert one POP compound 2,4,5-trichlorobiphenyl (PCB29). The fungi were grown in marine medium and their biomass was used as a biocatalyst in resting-cell reactions, aiming in the degradation of PCB29. The most potent microorganisms will further be explored in order to elucidate the degradation pattern that leads to less toxic metabolites.

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P25-10

Biodegradation of diethyl phthalate (DEP) by marine bacteria

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Phthalates esters (PAEs) are a class of compounds derived from phthalic acid, used as plasticizers in the industry of many products due to their chemical properties that confer flexibility and durability. They are widely used in plastics, lubricants, solvents, clothing, cosmetics and can easily migrate into the environment from plastic products and other related products once not chemically bound to the polymeric matrix. Many works have revealed that PAEs and their metabolites are carcinogenic and responsible for endocrine disruptions, even at very low concentrations. Therefore, the environmental protection agency of many countries has classified most PAEs as emergent pollutants. PAEs have been detected in various environments, but the huge amounts of plastics and microplastics in oceans is especially worrisome. So, it is critically important to find mechanisms to efficiently remove PAEs from the environment. In this context, in the present study, DEP-degrading marine bacteria were enriched from environmental marine samples (water and sediments) and the DEP degradation potential was investigated. Two different strains were isolated and reported to degrade DEP until 200 mg/L as only carbon source in a liquid mineral medium culture. The strains were identified by mass spectrometry (MALDI-TOF) as *Burkholderia cepacia* and *Ralstonia picketii*. Some studies about bioremediation of phthalates has already been carried out, and although little is known about the degradation capacity of PAEs by marine bacteria, this method has shown to be very promising for the remediation of these compounds in other environments.

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P25-11

Partial characterization of esterases from *Fusarium culmorum* grown in media containing di (2-ethyl hexyl phthalate) in solid-state and submerged fermentation

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Di (2-ethylhexyl) phthalate (DEHP) is a plasticizer used in the polyvinyl chloride (PVC) industry. The indiscriminate use of various products manufactured with PVC, causes this plasticizer to be considered a contaminant. *Fusarium culmorum* is a phytopathogenic fungus that has the ability to produce esterase enzymes. Esterases are of great importance because they can break the ester bonds present in the plasticizers. In this work, the activity of esterases produced by *F. culmorum* grown in media supplemented with different concentrations of DEHP (1500 and 2000 mg/L) in solid-state fermentation and submerged fermentation was characterized by biochemical tests and polyacrylamide gel electrophoresis. *F. culmorum* showed the highest esterase activity in media supplemented with 1500 and 2000 mg DEHP/L in solid-state fermentation. A great number of esterase activity bands (isoenzymes) were observed in the DEHP-supplemented media, having a molecular weight of about 20, 25, 37, 45, 55, 75 and 150 kDa, in both fermentation systems. 1500 mg of DEHP/L induced a high production of esterases, demonstrating that high concentrations of DEHP did not inhibit the enzymatic production of the fungus.

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P25-12

Effect of the pH on growth and esterase activity of *Fusarium culmorum* grown on media supplemented with di (2-ethylhexyl) phthalate in submerged fermentation

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Di (2-ethylhexyl) phthalate (DEHP) is a plasticizer widely used in the manufacture of plastics, and it is an environmental contaminant. *Fusarium culmorum* has shown ability to degrade DEHP due its esterases production. Cultivation conditions are essential in successful enzyme production by the organism, that's why optimization of the pH is crucial in the fermentation process. This fungus was grown at different pHs (5.5, 6.0, 6.6, 7.0, 7.5, 8.0, 8.5 and 9.0) in a medium added with DEHP (initial concentration 1000 mg/L) as sole carbon source at 25 °C for 228 h in submerged fermentation. In this work, the influence of pH on the specific growth rate, maximum biomass, esterase activity (evaluated by biochemical tests and polyacrylamide gel electrophoresis) and enzymatic yield parameters for *F. culmorum* were determined. It was found that the greatest kinetic growth and enzymatic yield parameters were observed at a pH of 6.5. Four esterase activity bands (isoenzymes) were observed in the DEHP-supplemented media, having a molecular weight of about 20 kDa, 25 kDa, 37 kDa and 50 kDa approximately. In general, the bands were observed between 72 and 228 h. These studies showed that 6.5 was the optimum pH for growth and esterase production of *F. culmorum*.

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P25-13

Enzymatic depolymerization of polyurethanes for biorecycling process

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Polyurethanes (PU) are synthetic polymers intended for long term applications such as isolation panels for construction or furniture. PU are specifically designed to resist against environmental factors such as climate constraints (low and high temperature, moisture), abrasion and microbial attack (biotic and abiotic degradations). These resistances lead to the pervasive spread of this material in the environment. In combination with other plastic materials released, it may exert negative and unpredictable effects on both aquatic and terrestrial ecosystems. Biochemical recycling appears as a promising solution for PU waste management. Specific enzymes are able to depolymerize/deconstruct polymers to release chemicals. These chemicals can then be used as building blocks to synthesize new macromolecular architectures.

In the frame of our study, a collection of hydrolases was screened on two model urethane substrates leading to the selection of an amidase able to cleave the urethane bond and an esterase able to hydrolyze a polyester PU dispersion. Enzymatic activity was then evaluated on four thermoplastic PU (TPU). The highest activity was measured for the esterase on a polyester PU with 33% weight loss after 51 days of incubation at 37 °C. Deep cracks on the polymer surface and the presence of oligomers in the remaining TPU pieces confirmed the high enzymatic efficiency. The corresponding main degradation products were identified to understand the scission mechanism. Combining the esterase and the amidase also led to a significant hydrolysis of this polyester PU. Specific degradation products were detected revealing the efficiency of the urethane bonds hydrolysis.

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P26-1

Reactive extraction of 6-aminopenicillanic acid

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6-Aminopenicilanic acid (6APA) is the main component of semi-synthetic Penicillins, antibiotics that are obtained by 6APA acylation and formation of amidic bonds different from natural ones. 6APA is the biosynthetic product of some Penicillin producing fungus grown on nutritive media without precursors. Because the biosynthesis process is economically inefficient, some chemical or enzymatic methods for Penicillin G hydrolysis to 6APA were proposed on industrial scale. The solution obtained by enzymatic hydrolysis of Penicillin G contains about 4–5% 6APA, 1.8–3% phenyl acetic acid (PAA) and 0.8–1% unhydrolized Penicillin G (PG). Whereas the industrial separation of 6APA needs large amounts materials and high energy consumption for extraction and acidification processes, the aim of this work was to establish the conditions for the selective separation of 6APA by reactive extraction from mixture obtained by enzymatic hydrolysis of Penicillin G. In order to select the most efficient extraction system, the individual extractions of 6APA with organophosphoric acids (di-2-(ethylhexyl) phosphoric acid, D2EHPA) and high molecular weight amines type (lauryl-trialkyl-methyl-amine, Amberlite LA-2) extraction agents were studied. The results indicated that by increasing the pH value of the aqueous phase (pH > 6–7) the extraction degree obtained with Amberlite LA-2 was significantly higher compared to those obtained for physical or reactive extraction with D2EHPA. Using the influences of the pH value of the aqueous phase and the concentration of Amberlite LA-2 in 1,2-dichloroethane on selective separation of a mixture of 6APA, PG and PAA, 6APA was selectively separated at pH = 10, the overall extraction degree being 98.8%.

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P26-2

Partitioning of bromelain enzyme extracted from *Ananas comosus* in different PEG–salt–water aqueous two phase system

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Aqueous two-phase system (ATPS) is suitable for purification of enzymes and proteins by preferentially partitioning the desired biomolecule in one phase and interfering substances into another phase. The biocompatibility of ATPS provides a very low interfacial tension between the phases, which results in high mass transfer and ease of scale up. Bromelain is the denomination given to the group of endoproteases obtained from members of the Bromeliaceae family. The objective of the present study is to relate the main studies in India that has proven that bromelain purification can be cost-effective, in addition to the well-known benefits owned by such enzymes, and highlight the applications that create their market potential in the World.

In the present work, partitioning of bromelain enzyme, extracted from the peel and crown leaves of pineapple fruit, in different PEG/salt two-phase systems was studied. ATPS comprising different compositions of PEG X (X = 4000, 6000)/salts (ammonium sulfate, sodium sulfate, potassium phosphate and trisodium citrate) were formed and characterized with the help of binodal curve and tie-line length. The specific activity of Bromelain in the crude extract of peel and crown was 0.15 U/mg and 0.25 U/mg respectively, and Specific activity after purification using ammonium sulfate precipitation, dialysis and ATPS (PEG 4000-Ammonium Sulfate-Water system) was 1.66 U/mg and 12 U/mg for peel and Crown respectively. Final fold purification of peel and crown is 11.06 and 48 respectively.

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P26-3

Recycling of ionic liquids used for an efficient pretreatment of lignocellulosic materials

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In the last decades, a flourishing interest in the utilization of ionic liquids for the processing of biomass is evident. These neoteric solvents, labeled as greener, have been found to be able to fractionate lignocellulosic materials. In this research work, we have bet for matching chloline cation (Ch) and glycine (Gly) anion as lignin solubilizing agent and the obtained results have been promising. However, taking into account the cost associated with the IL synthesis, its recovery and recycling is a key factor for industrial utilization in the light of environmental and economic concerns. Consequently, the subsequent challenge was to separate ChGly from the delignification stage in order to reuse it. Therefore, a volatile organic solvent has been added to the lignin-fraction under stirring conditions at 45 °C for one day. After this, the sample was kept to settle and two fractions were obtained: a dissolved part formed by lignin-organic solvent and the ionic liquid undissolved part. The latter fraction was submitted to vacuum evaporation and analyzed by ATR-FTIR and GC analysis. The information from these data suggests that ChGly remained in the bottom aqueous phase and the lignin content was dissolved in the organic upper phase. Consequently, the recovered bio ionic liquid, after a previous dried stage, was reused in different cycles to evaluate its efficiency. The results presented in this work make up the basis for a rational design of bio-ILs for delignification of lignocellulosic materials. Acknowledgements. We are grateful to the Spanish Ministry of Economy and Competitiveness for the financial support of this work (project CTQ2015-71436-C2-1-R), which has partial financial support from the FEDER funds of the European Union.

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P26-4

Withdrawn

P26-5

Isolation, purification and physicochemical characteristics of vitamin K2 from Flavobacterium

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Menaquinones (MK-n), that is, MK-4, MK-5, and MK-6, can be produced by the fermentation of *Flavobacterium meningosepticum*. This article deals with the process of extraction, purification and identification of the menaquinones. Among the tested organic solvents, the maximum extraction yields of the menaquinones were obtained by using methanol, reaching up to 99.1% when extracted for three times and 20 min each time. A series of chromatog-raphy including macroporous adsorption resin chromatography, gel permeation chromatography and reversed-phase C18 silica gel chromatography with large processing capacity from 0.95 to 7.0 mg menaquinones per gram packing were applied to the purification and separation of the menaquinone homologs in the crude extract. The purity of MK-5 and MK-6 crystallization could reach up to 96.3%and 99.2% calculated by external standard method (98.0% and 99.3%calculated by peak area), respectively. A high recovery ratio of MK-4, MK-5 and MK-6, that was, 94.7% (not crystallized), 87.3% and 88.2% were gained in the whole process from extraction to crystal-lization. Throughout the whole process, only two organic solvents containing methanol and dichloromethane were used, which was conductive to the recovery of the organic solvents and applica-tion on large-scale. The menaquinone homologs were identified as MK-4, MK-5 and MK-6 by MS, IR and 1H-NMR. DSC and TGA analysis demonstated that the melting points of MK-5 and MK-6 were 39.44 °C and 47.92 °C, and the boiling points were 223.94 °C and 233.81 °C, respectively.

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P26-6

Aqueous two-phase systems as an alternative to chromatography? Characterization of a PEG-citrate system for antibody purification

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Monoclonal antibodies and related products have become the dominant product class in the biopharmaceutical industry. As a result of constant efforts, the originally low product titers have increased over the years and are often approaching 10 [g/L]. This rise in the productivity of mammalian cell-based processes has led to downstream processing having difficulty to handle such large quantities at a reasonable cost. Alternatives to Protein A affinity chromatography are thus being sought and extraction in aqueous two-phase systems (ATPS) are among the most promising.

Our experimental model was an IgG1 type antibody present at a concentration of 3.6 [g/L] in a clarified supernatant of mammalian cell culture. We have investigated its partition in a polyethylene glycol (PEG) 4000/sodium citrate ATPS, which phase diagram was first characterized by turbidimetric titration. The measured binodal curves and the tie lines were successfully described by simple models. Using a Stavex® -generated experimental plan, optimal conditions were identified for the extraction of IgG1. In a mixture containing 8% citrate, 15% PEG 4000, 40% fermentation medium and no NaCl at pH 6.0 (the rest being water), 95% of the antibody were collected in the bottom (citrate-rich) phase at a concentration 1.7 times that of the initial sample.

The extraction was then performed continuously in a microfluidic device. However, the low values of Reynolds number achieved there prevented the establishment of partition equilibrium and recovery yields were lower. Ways to improve these results have been tested and led to the design of a new micromixer.

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P27-1

Production of double-stranded RNA in chloroplast of *Chlamydomonas reinhardtii* for feed-delivered suppression of White Spot Syndrome Virus (WSSV) in shrimps

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White Spot Syndrome Virus (WSSV) is a major cause of shrimp mortalities leading to large-scale economic losses in the aquaculture industry. Double-stranded RNAs (dsRNAs) with homology to viral gene sequences have been produced using bacterial hosts and, when fed to shrimps, were shown to initiate post-transcriptional silencing of the targeted viral genes and protect the shrimps from viral infection. To avoid the use of bacterial hosts which may provoke environmental and food safety concerns, this study aimed to test the feasibility of producing dsRNA specific to the *VP28* gene encoding a structural protein of WSSV in the chloroplast of microalga *Chlamydomonas reinhardtii*. A partial coding sequence of the *VP28* gene was inserted in-between two convergent *T7* promoters, which resulted in production of both sense and anti-sense transcripts of the gene sequence. To prove functionality of the convergent promoters, the dsRNA production was first tested in *Escherichia coli* (DE3). The dsRNA yield, after DNase and RNase A treatment was approximately 11.7 ± 1.9 μg/mL bacterial culture. The chloroplast-expression vector was successfully constructed by transferring the *VP28* gene sequence to the pSRSapI plasmid containing two convergent *PsaA* promoters. Glass bead-mediated transformation into microalgal cells had been attempted 6 trials using 5–8 μg of the obtained plasmid and Tris-acetate-phosphate culture of *C. reinhardtii* strain TN72, which originally lacked photosynthesis capability due to the *PsbH* gene deletion. Selection of the transformants was performed under a photoautotrophic condition, due to the restoration of the photosynthesis activity as a result of the *PsbH* gene present on the expression plasmid, in Sueoka's High Salt medium. However, no transformant has yet been obtained for an unknown reason.

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P27-2

Autophagy stimulation in the light of degradation efficiency of glycosaminoglycans in mucopolysaccharidosis type III

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Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases caused by absence or malfunctioning of enzymes involved in the degradation of glycosaminoglycans (GAGs). In a healthy organism, GAGs degradation takes place in lysosomes due to the activity of several enzymes that sequentially remove individual mono-sugars or chemical groups from GAG chains. The sequential way of degradation causes that the defect of one of the enzymes prevents the activity of the others. Therefore, degradation of GAGs stops at various stages of this process, and partially-degraded molecules accumulate in the lysosomes. Recent studies have shown that one of the currently tested therapeutics - genistein - can lower the level of GAGs storage in cells by increasing the biogenesis of lysosomes. Lysosomes, in the autophagy process, can degrade the accumulated GAGs using enzymes produced within their own structures. Autophagy, induced by various natural compounds, is considered one of the most promising therapeutic strategies in many diseases related to accumulation of various molecules. How-

ever only studies with genistein in MPS have made it clear that this process can also be useful in degradation of accumulated GAGs. In this study, we have tested various compounds, known to induce autophagy through different pathways. We found that genistein, trehalose, curcumin, capsaicin, resveratrol, and calcitriol differentially affected levels of GAGs in MPS III cells. These results are considered in the light of a possible use of the tested compounds in potential therapy for MPS III, either alone or in combinations of two or more of them.

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P27-3

A dual-type L2 11–88 peptide from HPV types 16/18 induced strong immune responses and broad spectrum cross-reactive antibodies in mice

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E. coli-derived concatenated, multitype L2-conserved epitopes of human papillomavirus (HPV) L2 protein might represent a less expensive and pan-type vaccine alternative (compared to type-specific HPV L1 virus-like particles), if stable protein expression and strong immunogenicity features could be met. Herein, three dual-type- (DT-) HPV L2 fusion peptides comprising the three head-to-tail tandem repeats (multimers) of either HPV 16 epitope "17–36" or "69–81" or one copy (monomer) of 11–88 fused to the same residues of HPV 18 were constructed and expressed in *E. coli*. SDS-PAGE and Western blot analyses indicated the proper expression and stability of the *E. coli*-derived DT peptides. Mice immunized by formulation of the purified DT peptides and Freund's adjuvant (CFA/IFA) raised neutralizing antibodies (NAbs; the highest for DT: 11–88 peptide) which showed proper cross-reactivity to HPV types: 18, 16, 31, and 45 and efficiently neutralized HPV 18/16 pseudoviruses in vitro. Immunization studies in mice by formulation of the DT: 11–88 × 1 peptide with various adjuvants (alum, MF59, and Montanides ISA 720 and 50) indicated that Montanide adjuvants elicited the highest cross-reactive titers of NAbs and similar levels of IgG1 and IgG2a (switching towards balanced Th1/Th2 responses). The results implied development of low-cost E. coli-derived DT: 11–88 peptide formulated in human compatible ISA 720 adjuvant as a HPV vaccine.

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P27-4

Tamoxifen resistance breast cancer induces mesenchymal phenotype in fibroblasts through TGFβ1 signalling

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Introduction: TGFβ1 is a known inducer of epithelial to mesenchymal transition (EMT) in breast cancer cells. But the effect of this cytokine on fibroblasts in breast tumour stroma is least apparent. Here we deciphered the role of TGFβ1 from parental MCF-7 and its resistant counterpart in promoting cancer associated fibroblasts (CAF) phenotype through EMT.

Methodology: Chemo-resistant cell lines were established by treating MCF-7 cell lines with sublethal dose of Tamoxifen over several cycles and characterized by MTT assay. Western blot (WB) and ELISA were carried out for quantification of TGFβ1 expression in both MCF-7 (sensitive) and MCF-7 TAM (resistant) cells. Conditioned media (CM) from both the parental MCF-7 and Tamoxifen resistant MCF-7 (MCF TAM) cells was collected and treated on Normal Fibroblast (NF) in a time and dose dependent manner. Initial screening for the expression of CAF markers Caveolin-1 and α-SMA was carried out by qRT PCR and further confirmed by WB. EMT markers post CM treatment was assessed by WB analysis.

Results: TGFβ1 expression was remarkably high in MCF-7 TAM as compared to MCF-7. MCF-7 TAM CM promoted CAF phenotype with downregulation of Cav-1 and upregulation of α-SMA expression in NF. Alongside, expression of EMT markers were greatly elevated in NF.

Conclusion: We conclude that tamoxifen resistant breast cancer cells can induce cancer associated fibroblast like phenotype in normal fibroblast. The mechanism underlies the induction of EMT markers in fibroblasts through TGFβ1 signalling.

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P27-5

Polyploid giant cancer cells induce growth arrest and cytoskeletal rearrangement in breast cancer cells

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Triple negative breast cancers are known to be the most aggressive form of malignant tumors with poor prognosis. Polyploid giant cancer cells (PGCCs) derived from chemoresistant population have not been given their much deserved attention over the past few years. Our observation suggests that PGCCs play a very significant role in the regulation of tumor microenvironment. After treatment with the conditioned media of polyploid giant cells, the proliferation of triple negative breast cancer cell lines MDA MB 231 and MDA MB 468 was found to be reduced in a dose dependent manner as well as the morphology was found to be altered. Deregulation in the levels of cyclins and cyclin-dependent kinases in the cell line MDA MB 231 was confirmed by quantitative RT-PCR and western blot analysis. The rhodamine phalloidin-DAPI stained images of cell line MDA MB 231 showed a reorganization of actin filaments. Quantitative RT-PCR analysis of genes fascin and Ena/Vasp confirmed their overexpression in PGCC conditioned media-treated MDA MB 231. A cell cycle analysis was performed on both the cell lines MDA MB 231 and MDA MB 468 after treatment with the conditioned media of PGCCs. The results show that there might be a cell cycle arrest at the G1 phase. As a whole, we report here that the polyploid giant cancer cells reduce the proliferative ability by altering the G1 phase, and change morphology by actin remodeling of the neighboring cancer cells.

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P27-6

Targeting ECM via stress associated regulation: A potential therapeutic avenue for overcoming temozolamide resistance in glioblastoma

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Glioblastoma multiforme (GBM) is the most aggressive form of glioma with poor prognosis. The most common treatment regimen includes the use of a chemotherapeutic DNA methyltransferase

inhibitor temozolomide (TMZ), in conjunction with surgery and/or irradiation. Resistance to TMZ poses a major threat to the alleviation of this disease necessitating the need for development of novel drug targets. Hence in this study, TMZ resistant glioma cell lines were developed and characterized in order to explore alternative therapeutic approaches. Upregulation of the anti-oxidative stress pathways contribute towards the development of resistance. A co-relation between oxidative stress related genes like ROS, HSP70, HIF-1 α, NFE2L2 and extracellular matrix (ECM) degrading enzymes like MMP2, MMP3, MMP9 etc. was studied and a positive association was found. Hence a specific oxidative stress gene modulator S-Nitroso-N-acetylpenicillamine (SNAP) was used along with TMZ to therapeutically tackle TMZ resistance. SNAP, an S-nitrosothiol and may be involved in the modulation of oxidative stress due to its nitric oxide releasing property. Co-administration of SNAP and TMZ exhibited a significant increase in cell apoptosis and inhibition of cell growth in vitro. Downregulation of EMT, ECM markers, anti-apoptotic markers like Bcl-Xl and Bcl-2, VEGF and enhanced expression of pro-apoptosis markers like BAX, BAD, cytochrome c were also observed. Thus, our result shows that this combination chemotherapy is more effective for TMZ resistant cells. Hence, our observations for the first time provide a new insight for the development of novel therapies for overcoming TMZ resistance in GBM.

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P27-7

The study of the proteinases of *Sarocladium strictum*, a new producer of complex trombolytic compounds

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In the twenty-first century cardiovascular diseases and their complications assumed the epidemic character, mostly affecting developed countries. In this regard, medicine is increasingly in need of new plasminogen activators, which are actively used in the treatment of various blood disorders. However, the majority of such drugs have significant shortcomings, therefore, the development of new compounds is an extremely urgent task.

A very promising candidate is the complex of thrombolytic enzymes strictoliase, produced by the micromycete Sarocladium strictum. When strictoliase was applied to fibrin plates, according to Astrup-Müllerz method, plasminogen activator activity and fibrinolytic activity were revealed. For further study of the proteinases properties, was performed the isoelectrofocusing. As result, were found four fractions, which possessed high activity against the studied chromogenic peptide substrates that were similar to the proteins of the hemostasis system.

Protease of peak I showed a narrow substrate specificity and pronounced urokinase activity, which was 174.8 μmoles pNA/ml min. Protease II and III peptides also showed high urokinase activity, which were 89.3 and 78.8 μmoles pNA/ml min, respectively, but were also able to hydrolyze other substrates thrombin, plasmin and tissue plasminogen activator substrates. Proteinase IV peak actively cleaved all the above listed substrates. Due to these activities it may be possible to use strictoliase it for the treatment of thrombophlebitis and phlebothrombosis. The use of the purified proteinases of strictoliase separately can make this drug more specialized, which can allow using the preparation as a thrombolytic agent or as a diagnostic for the concentration of plasminogen in blood.

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P27-8

Cytotoxic activity on *Leishmania infantum* promastigotes of a disintegrin isolated from *Cerastes cerastes* venom

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Investigation for new antimicrobial and antiparasitic compounds from Viperidae venoms represents a therapeutic alternative to existing drugs. Leishmaniasis, classified as a neglected tropical disease, occurs in many countries and still continues to cause significant morbidity and mortality in the developing world. This study reports the isolation and characterization of a disintegrin from Cerastes cerastes venom, exhibiting antiparasitic activity on *Leishmania infantum* promastigotes. This disintegrin, referred to Disintegrin_Cc, was isolated by two chromatographic steps on Sephadex G75 and an ion exchange chromatography on DEAE Sephadex A50 and then the active subfraction F2D3 was separated on a C8 column of RP-HPLC. The main eluted active peak was analyzed by MALDI TOF/TOF mass spectrometry. SDS-PAGE analysis indicated that this disintegrin was homogenous. Disintegrin_Cc induced 84.75% of parasiticidal activity and deep morpho-physiological alterations on the promastigote form of the parasites, in vitro. Disintegrin_Cc inhibited 80% of arachidonic acid-induced platelet aggregation. Mass spectrometry analysis indicated that this dimeric disintegrin of 14 193.97 Da contains an RGD domain and four intramolecular disulfide bridges. The isolated disintegrin presents a high percentage of identity with other related snake disintegrins. Predicted 3D structure indicated that this peptide shares partial homology with well-known active antimicrobial peptides. The obtained results suggest that the isolated molecule could constitute a promising therapeutic tool against this protozoan parasite associated disease.

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P27-9

Verification of the potency testing method for the monoclonal antibody drugs using reporter gene assay

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The market share of biopharmaceuticals in the entire pharmaceutical market is continuously increasing, and among them, therapeutic monoclonal antibodies (mAbs) have been most actively developing. However, due to the nature of the complicated structure, it is difficult to analyze its characteristics and quality. Particularly, in order to reflect the mechanism of action of an mAbs, a test using cell-line is mainly used in a potency test. In general, a particular cell line that responds to the product is selected and the evaluation of cell death or cell proliferation as a final endpoint.

It results in a large variation of the test result and it takes a long time of at least 3–4 days to complete. Therefore, it is required to develop a new test method, which can confirm the activity of the mAb in a more precise, fast and timely manner. Therefore, we tried to develop a reporter gene assay, which can confirm the potency of mAbs more quickly and accurately. In particular, we investigated the conditions under which the activity of the anti-TNFα antibody drug can be measured using a cell line, which was stably transfected with a reporter gene containing upstream transcription factor response elements of the TNFα signaling pathway. As a result, when NF- κB-RE-luc2P HEK293 cells were plated in a medium of neutralized TNFα, the IC50 was 68.5 ± 7.2 ng/mL for Infliximab. The relative standard deviation value was less than 4.0% when performing three experiments for precision test. The time required for the test was 1.5–2 days, and it took less time than the normal cell death/cell proliferative activity assay.

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P27-10

IgY-Technology: Preparation and potential application in the treatment of scorpion envenomation

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In our country, scorpion envenomation incidence is attributed mainly to *Androctonus australis* hector (Aah) scorpion venom. The produced antivenom (IgG) from mammalian blood is right now controversed and may cause various clinical side effects. The aim of this study is to develop an alternative antivenom antibody (immunoglobulin Y–IgY) from laying hens.

IgY in egg yolk from hens previously immunized with Aah venom was extracted by water and precipitated by ammonium sulfate. IgY was identified by SDS-PAGE, ELISA and Ouchterlony, its neutralizing assay was conducted on a murine experimental model. The protective efficacy of IgY on the pathophysiological effects and the inflammatory response induced by Aah venom was assessed by tissue and metabolical analysis in the liver and heart. The inflammatory response was evaluated by the measurement of the granulocyte tissue infiltration and oxidative/nitrosative status.

Results revealed a high antibodies titer of IgYs to their immunogen (Aah venom). Overall, IgYs efficiently protected mice from envenomation and neutralized the lethal effects of scorpion venom with a good efficacy; the median effective dose (ED50) was 221 μL/2LD50. These IgY antibodies could prevent severe pathological effects and inflammatory response induced by scorpion venom.

In conclusion, IgY antibody response was successfully conducted in laying hens injected with Aahvenom. IgY against Aah venom was obtained for the first time, and it exhibited strong neutralizing potency on mice.

Laying hens could be used as an alternative source of polyclonal antibodies against Aah venom due to several advantages as compared with mammals traditionally used for such purpose.

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P27-11

Inhibitors of RANKL-induced osteoclast differentiation from the Marine Fungus *Aspergillus flocculosus* isolated from a Sponge *Stylissa sp.*

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Large numbers of novel secondary metabolites from the ocean have structural diversities and potent bioactivities. Many marine organisms, especially microorganisms, have been attracting significant attention from natural product chemists and biologists due to the potential of marine natural products as drugs during recent years. As part of our continuing efforts to discover bioactive natural products from sponge-derived microorganisms, various marine sponges were collected and investigated for the isolation and identification of microorganisms. During studies on the diversity of fungi, a marine fungal strain 01NT-1.1.5, *Aspergillus flocculosus* derived from a sponge *Stylissa* sp., was isolated through experimental tools for the isolation of microorganisms. The crude extract of the strain was purified further by a reversed-phase HPLC to yield one new compound, ochraceopone F (1), and four known compounds aspertetranone D (2), cycloechinulin (3), wasabidienone E (4), and mactanamide (5). The structures of the known compounds were identified by 1D and 2D NMR analysis and comparison with literature data. All compounds were tested for anti-proliferative activity on human cancer cell lines and RANKL-induced osteoclast differentiation inhibitory effect using a TRAP assay. Among compounds 1-5, mactanamide (5) showed potent RANKL-induced osteoclast differentiation inhibitory effect.

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P27-12

Long-acting anti-diabetic peptide drug based on genetic fusion with an albumin-binding aptide

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Despite the therapeutic potential of exendin-4 as a glucagon-like peptide-1 (GLP-1) mimetic for the treatment of type 2 diabetes, its utility has so far been limited because of the low level of patient compliance due to the requirement for frequent injections. In this study, novel long-acting fusion protein as exendin-4 analog was produced by fusion to human serum albumin-specific aptide. Aptides, developed by our group, are a novel class of peptides that has a high affinity and specificity against target molecule. Phage display of aptide libraries allowed us to screen and isolated an aptide (APT_{HSA}) specific for human serum albumin that showed high target affinity and specificity. The APT_{HSA} exhibited an association rate constant ($k\alpha$) of ~1.65×10^5 $M^{-1}s^{-1}$ and a dissociation rate constant (kd) of 2.11×10^2 s^{-1}, yielding a dissociation constant (KD) of ~128 nM. The fusion peptide, Exenatide-APTHSA, was prepared by protein expression and purification. Unlike other fusion proteins or peptides between exenatide and albumin-binding

affinity molecules, the resulting fusion peptide showed similar glucose-induced insulin secretion activity to native exenatide when tested *in vitro* using the INS-1 cell line. Pharmacokinetic study after subcutaneous administration of the fusion peptide revealed that it showed a 4-fold longer plasma half-life compare to exenatide from 0.35 h to 1.3 h. Furthermore, the exenatide-APT_{HSA} fusion peptide showed significantly improved anti-hyperglycemic effect in oral glucose tolerance test (OGTT) and hypoglycemic effect than did exenatide in a db/db type 2 diabetes mouse model. These results suggest that the exendin4-APT_{HSA} fusion protein could be used as a potential anti-diabetic agent for the treatment of type 2 diabetes.

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P28-1

Understanding the heterologous expression of HIV-1 Env glycoproteins in CHO Cells

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Background and objectives: Soluble native-like trimers (NLT) of the HIV-1 envelope glycoprotein (Env) applied as antigens are among the most promising approaches to elicit a broadly neutralizing antibody response fighting HIV-1 infection. In this project, we aim to determine factors influencing the efficient production of Env protein variants in CHO cells for clinical grade protein production.

Methods: Stable CHO cell clones co-expressing variants of HIV-1 Env and human furin pro-protein convertase (mediates efficient cleavage of the Env precursor) were generated by BAC vector technology (Zboray et al., 2015). Screening for trimeric Env specific expression of clonal populations was performed by ELISA considering clone specific growth behaviour. The top producers were evaluated under lab-scale production conditions to identify the best clone for large scale antigen manufacture.

Results and conclusion: CHO cell lines expressing a furin cleaved Env variant and a non-cleaved Env variant were generated. Characterisation by Native PAGE, SDS PAGE and Western blot analysis of affinity purified Env proteins indicated specific signals for the respective proteins at the expected molecular mass. SE-HPLC showed only minor fractions of non-trimeric forms. Additionally, this could be confirmed by negative-stain EM where all particles assayed could be classified as native-like trimers.

To gain a better understanding of factors influencing the intracellular processing of HIV Env in a heterologous expression situation is of great interest. Thus, further investigations will address to what extent the role of high level furin co-expression may or may not impede increased Env protein yield.

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P28-2

Quality matters – Is IgM production influenced by external process conditions?

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Immunoglobulin M (IgM) antibodies are reckoned as alternative immunoglobulin formats for prospective therapy as well as diagnostic approaches. Currently they are used as a marker for several infectious diseases, but are also investigated in clinical trials as therapeutics for cancer and autoimmune diseases. Nevertheless, clinical progress is limited due to challenges in recombinant production and downstream processing. The recombinant expression hosts need to connect lots of disulfide bonds, perform proper post-translational modifications as well as secrete the correctly assembled polymer into the culture supernatant.

We established stable producing CHO DG44 cell lines and studied how extracellular factors like cultivation temperature and pH affect the recombinant IgM production. Cell growth and productivity as well as quality criteria were investigated. Polymer distribution was quantified directly in cell culture supernatant and in purified product with densitometry and size exclusion HPLC. Glycans were identified with fluorescence labeling and HPLC coupled to mass spectrometry.

Using a design of experiment approach, we found that cultivation temperature has an impact on peak cell density and IgM titer, whereas the pH of the culture broth has only a minor impact. Quality attributes like polymer distribution and glycosylation remained unaffected by both external factors.

Concluding our results, the established CHO DG44 cell line produced IgM with a consistent product quality over a range of temperatures and pH values. Moreover temperature and pH seem not to be the cause of potential product variety during a production process.

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P28-3

Determination of CHO biomass composition

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Chinese hamster ovary (CHO) cells are the primary host organism for the production of protein biopharmaceuticals. Significant improvements in product yield and cell growth were achieved in the past years by bioprocess and media optimization, directed evolution and targeted genetic engineering. However, a deeper understanding of the underlying processes in the cells is still limited. Recently, for the first time, a CHO-specific genome scale metabolic model was created in a large community effort. This model is a comprehensive resource of CHO metabolism. Using metabolic modeling toolsets, we are now starting to get valuable insights into the cells' metabolism, their protein production capabilities and potential limitations. One essential input for the model is biomass composition. It has been shown that using strain and condition specific biomass together with bioprocess data improves the model's predictions. Currently, however, the model uses estimates and literature values, since comprehensive data about CHO cell composition, specifically of individual cell lines or strains, are lacking. In this work, methods for the determination of CHO biomass composition (proteins and amino acids, lipids, DNA, RNA and cell dry mass) were established. These include chromatography and mass spectrometry determination of amino acids, fluorimetric and spectrophotometric quantification of nucleic acids and gravimetric quantification of cell dry mass. Biomass compositions of various CHO cell lines, both hosts and producers, are being evaluated throughout batch cultures to answer the question whether a generic CHO composition is sufficient for modelling or whether it is necessary to use strain or condition specific biomass data.

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P28-4

Identifying new engineering targets in Chinese hamster ovary cells

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Higher producing CHO cells are an important contribution to pharmaceutical production. Finding new genetic targets may be achieved by screening gene knock downs (KD) using small interference RNA (siRNA) libraries. Despite its importance for industry, currently no siRNA library for CHO exists. Applying a mouse siRNA library to a GFP expressing suspension CHO cell line yielded two possible nucleolar protein targets for productivity enhancement: CHD4, which is part of the nucleosome remodeling and deacetylase complex (NuRD), and RAD21 that is involved in the repair of DNA double strand breaks and is additionally associated with mitotic chromatin.

KD of the genes led to variable results: in two tested cell lines CHD4 – KD led to reduced growth, but higher specific productivity (qP). In a third cell line the KD did not affect cell growth, but reduced qP. RAD21 – KD showed three different responses in as many cell lines: (i) no effect (ii) improved qP, but reduced growth (iii) improved qP, similar growth rate as in mock.

These results partly confirm the screening results, but also demonstrate the large differences in behavior in CHO cell lines. An explanation would be the endogenous expression of the respective genes in the different cell lines. Thus engineering approaches to improve phenotypes may have to be adapted to different CHO lineages or even subclones, depending on the starting transcriptome of each cell line.

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P28-5

A CRISPR/Cas9 based engineering strategy for overexpression of multiple genes in Chinese hamster ovary cells

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Manipulation of multiple genes to engineer Chinese Hamster Ovary (CHO) cells for better performance in production processes of biopharmaceuticals has recently become popular. Yet, identification of useful genes is a very time consuming task and unequivocal assessment of their effects in combination(s) on the cellular phenotype is difficult due to high variation between subclones. Here, we present the proof-of-concept of a novel strategy for overexpression of multiple genes in CHO cells, by multiplexable activation of artificially repressed genes (MAARGE). This strategy will allow faster screening of gene overexpression combinations. MAARGE, in its here presented instalment, comprises four genes of interest (fluorescent proteins) that can all be stably integrated into the genome in a single integration event. However, three of those genes are repressed by a repressor element (RE) that is integrated between promoter and translation start site. GuideRNA (gRNA) targets flanking the REs then allow to specifically delete the RE with CRISPR/Cas9 and thus to activate the expression of the corresponding gene(s). We show that both individual and multiplexed activation of the genes in transient and also in a stably transfected CHO cell line is possible. Also, upon transfection of a stable cell line with all three gRNAs, it was possible, in a single experiment, to isolate cells that express all potential gene combinations. For pathway and cell engineering studies, the selected genes can be expressed linked to these fluorescent genes (e.g., via an IRES) and cells with the desired co-expression pattern sorted, thus obviating the necessity to subclone for subsequent phenotypic characterization of the engineered cells.

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P29-1

Electrochemical impedance immunosensor for ferritin detection

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Ferritin was discovered in 1937 by French scientist Laufberger and isolated as a new protein from horse spleen. It is a ubiquitous iron–binding protein which plays an important role in the storage of intracellular iron. Ferritin is one of the most important markers of iron deficiency anemia, which is very common in human populations around the world. In addition, it is important as an acute phase reactant in many cases of inflammation and in some cases as a tumor marker. Due to these important properties, sensitive determination of serum ferritin levels is very important.

In this study, a new impedimetric immunosensor for the determination of ferritin was developed. As a basic principle, an affinity–based biosensor system based on antigen–antibody interaction was developed and the impedance changes of the sensing surface were observed by electrochemical impedance spectroscopy technique. Ferritin determination was carried out by way of the mentioned changes. Antigen–antibody interaction was carried out between the anti-ferritin antibody and the ferritin biomolecules. The sensitivity of the immunosensor system has been increased as a result of various modifications of the sensor surfaces used.

Anti-ferritin antibodies were covalently immobilized onto the gold electrode surface via a self-assembled monolayer (SAM) of 11 – mercaptoundecanoic acid (11 – MUA). Ferritin antigens were detected by electrochemical impedance spectroscopy (EIS) as a result of their interaction with surface-immobilized antibodies. Optimization and characterization studies were carried out and the linear range of the biosensor was from 2.5 to 400 pg/mL of ferritin.

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P29-2

Selection of ssDNA aptamers for the development of impedance biosensor to detect sarcosine

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Sarcosine is a potential prostate cancer biomarker that can be non-invasively analyzed in urine. Within the scope of this study, sarcosine-specific DNA aptamers were selected using graphene oxide-assisted systemic evolution of ligands by exponential enrichment (GO-SELEX). It was tested whether the developed aptamers could be used in biosensor applications. For this purpose, 1 μM aptamer solution was first immobilized onto the gold electrode surface through the thiol group at its 5'-end for 17 h. Hence, self-assembled monolayer (SAM) was created. Subsequently, the electrode was immersed into the 1 mM solution of 6-Mercapto-1-hexanol (MCH) for blockage of the remaining gaps on the gold surface. Finally, the immobilized aptamer was treated with increasing concentrations ($5.0 - 500 \times 10^3$ pM) of sarcosine. After each step, the electrode surface was washed with the binding buffer. Impedance and cyclic voltammetry measurements were performed. The success of each immobilization step and the concentration of sarcosine were determined. A significant response to sarcosine was obtained. Preliminary results show that the selected aptamer is promising for biosensor applications. Further studies to optimize and characterize the developed biosensor are in progress.

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P29-3

Molecular diagnosis of FXS in Indian MR cases

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Introduction: Fragile X Syndrome (FXS), the second most common type of X linked mental retardation (MR) is caused due to CGG repeat expansion mutation at FMR1 gene. The normal range CGG repeat range is 6–44, gray zone range 45–54 repeats, premutation range as 55–200 repeats and full mutation >200 repeats (affected). PM alleles are unstable and can expand to FM in subsequent generation. Thus tracing these individuals holds significance in alleviating the occurrence of FXS in the society and early diagnosis will help in improving the quality of life of affected subjects.

Objectives: Molecular characterization of FMRl gene in MR subjects by using TP PCR technique and identify carriers in the family tree was done.

Method: Genomic DNA was extracted from 201 subjects (141 males and 12 females) with unexplained developmental delay, intellectual disability and autism. TP-PCR amplification for FMR1 allele is conducted and amplicons generated were subjected to fragment analyses and results were documented.

Results: Thirteen (6.46%) full mutation were identified in 201 MR suspects (186 males and 15 females). Genetic counselling was given in all positive cases and family screening could be done in 9 of the full mutation males leading to identification of additional 15 PM (12 females and 1 males) 5 FM (1 females and 4 males) cases.

Conclusion: Present study validates use of TP PCR for routine testing of FMR1 mutation in clinical setup against use of southern blotting and available expensive commercial kits owing to its rapidity, sensitiveness and cost effectiveness.

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P29-5

TP-PCR mediated molecular screening for Fragile X carrier status in Indian women: Knowing the unknown

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Introduction: Fragile X Syndrome (FXS) is most common incurable X linked mental retardation caused due to FMR1 gene silencing due to hyper expansion and hyper-methylation of CGG repeats at 5′UTR. Expanded alleles include gray zone (45–54), premutation (55–200) and full mutation (>200) alleles. FXS carriers (premutation and grayzone) are present at high frequency in the general population (1 PM in 113–259 and 1 GZ in 66 females) and are at risk of transmitting expanded allele in subsequent generation giving rise to affected individuals. Screening women for FXS carrier status will open doors for genetic counselling and option of anti-natal diagnosis.

Objective: To detect FXS carriers in women of reproductive age by advanced TP-PCR method.

Methodology: Genomic DNA was extracted from 500 reproductive age women. After TP-PCR amplification, amplicons were subjected to fragment analyses and results documented.

Results: We detected 2 PM and 8 GZ carriers among studied cohort. Genetic counselling was given and family screening was offered, leading to further identification of 4 premutation and 1 gray zone carriers.

Conclusion: Establishing FXS mass screening program had greatly suffered due to unavailability of accurate, cost effective and rapid molecular techniques. Advanced TP-PCR developed by us is cheaper in comparison to available commercial kits and thus economically more feasible to be used in mass screening and FXS carrier detection.

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P29-6

CRELD1 gene variants leads to atrioventricular septal defects in Down syndrome

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Background: Congenital heart defects (CHD) are seen in around 40% of the Down syndrome (DS) patients. Atrioventricular septal defect (AVSD) or endocardial cushion defect is the commonest form of CHD in these children. CRELD1 gene is implicated in causation of sporadic AVSD.

Aims and objectives: In the present study, we evaluated the association and significance of CRELD1 variants with AVSD in Down syndrome (DS) patients.

Materials and methods: Sequencing was done in blood samples from 3 groups: group I (DS with AVSD), group II (DS without AVSD) and group III (non-syndromic AVSD cases).

Results: Twenty-two variants in CRELD1 gene were identified, comprising of sixteen novel and six previously reported variants. However, on the basis of sequence, as well as structure analysis, the variant c.973G>A(p.Glu325Lys) variant was identified only in DS

having AVSD group which was predicted to have significant effects on calcium binding of putative CRELD1 protein. Since CRELD1 gene acts as a regulator of calcineurin/NFATc1 signaling which is crucial for the regulation of cardiac development by dephosphorylation of the transcription factor, NFAT (nuclear factor of activated T cells), in cytoplasm, the variation in cb-EGF-like calcium binding domain in CRELD1 protein is likely to have pathogenic consequences.

Conclusions: Thus, we conclude that the CRELD1 gene is likely to have a major role in causation of AVSD phenotype in selected DS patients.

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P29-7

MicroRNA in situ hybridization with phosphate-methylated oligonucleotides (nDNA) probe

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MicroRNAs (miRNAs) are a cluster of small, non-coding RNA molecules, generally 18–22 nucleotides in length that play important roles in regulating gene expression by inducing mRNAs degradation. Previous studies have shown several correlations between aberrant miRNAs expression and a variety of human diseases. Typical miRNAs are considered as diagnostic and prognostic biomarkers. The need of developing highly sensitive and specific detection methods is necessary. In situ hybridization (ISH) is an important technique that provides both miRNA expression level and distribution in a single cell level. However, the main challenge of using DNA oligonucleotides as detecting probe is that the results lack of specificity since the small size and the nature fragile characterization of the target miRNA. In this study, we applied neutralized DNA (nDNA) modified oligonucleotides as ISH detecting probe. NDNA, a DNA analogue, with the backbone phosphate groups replaced by methylated groups, shows improved the hybridization properties due to the reduction of electrostatic repulsion between the double strands duplex. And the lipophilic character allows the probe transport through cell membrane easily. ISH were performed to visualize mimic exogenous miR-524-5p we transfected into HCT116 (human colon cancer cell lines) and the well-known endogenous oncomiR, miR-21 in colon cancer cell by 3′-digoxigenin (DIG) labelled nDNA modified probe. Through optimal design of the nDNA probe, the results demonstrated improved hybridization efficiency while remaining detecting specificity. Based on the success of applying nDNA probe on detecting miRNA through ISH, we expected the potential ability of nDNA modified oligonucleotides developing into theoretic agent.

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P29-8

Improvement of liver stiffness detected by FibroScan in patients with liver fibrosis after bariatric surgery

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Background and aim: Obesity is associated with various degrees of non-alcoholic liver disease (NAFLD) and secondary liver fibrosis. Literature data provides information regarding the improvement of liver structure after bariatric surgery. We aimed to follow-up the liver stiffness of 25 patients treated by gastric by-pass using FibroScan and to detect, if applicable, the improvement of the liver fibrosis stage.

Method: All patients were twice investigated by FibroScan measurement in Gastroenterology Department of Emergency Hospital of Constanta County, once before gastric by-pass intervention, and the second time, after 6 months from the surgery procedure. Liver biopsy was done in all patients in order to detect the accuracy of FibroScan.

Results: The liver stiffness measurement permitted to classify fibrosis stage of our patients according to number of kPa obtained before and after the surgical procedure. 16 patients (64%) had an improvement of liver stiffness and 5 of them (20%) had a reversal of fibrosis from stage 4 (>13.5 kPa) to stage 3 (<13.5 kPa). The statistic analyse showed a positive correlation between the liver stiffness improvement and the degree of weight loss ($r = 0.98$). Comparison with the gold standard procedure meaning the histological examination, showed a 100% concordance regarding the decrease of liver fibrosis staging.

Conclusion: Our study confirms that gastric by-pass has a good influence on liver structure damage. FibroScan is an accurate tool to use during follow-up management of obese patients with gastric by-pass in order to detect the liver histology improvement.

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P30-1

Immobilization and purification of soybean seed hull proteins of biotechnological interest identified by shotgun proteomic analysis

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Soybean crops have had a huge development. Soybean consumption is led by oil and flour, for which the hull must be removed. However, hull contains valuable proteins that can be extracted prior to pellet it for animal food. The aim of this work was the proteomic characterization of soybean seed hull aqueous extract as a way to identify candidate proteins for downstream processing, thus allowing the revaluation of this by-product of soybean manufacture. A hull extract was concentrated by ultrafiltration and subjected to analysis by nano-HPLC-ESI-Orbitrap. A total of 149 proteins were identified, among them 60 with potential biotechnological application, 15 in high abundance including peroxidase, urease and glycinin. These proteins were immobilized on chitosan mini-spheres. Peroxidase immobilization yield was over 90% when mini-spheres were derivatized with the triazine dye Reactive Blue 4, selected after a screening with 19 dyes. For urease and glycinin, mini-spheres were chemically modified to obtain thiosulfonate

derivatives, thus allowing the immobilization of urease (95% yield) and the purification of glycinin by elution with β-mercaptoethanol (70% yield). For immobilized urease, the pH of maximal activity shifted from 7 to 4–5, allowing its utilization in acidic media, i.e. decrease of urea concentration in wine. Thermal, operational and storage stability of the immobilized proteins were improved, as compared with the free enzymes. Immobilized peroxidase and urease were applied to solve real problems: degradation of phenols in wastewater and dosage of serum urea, respectively, along various cycles with no significant decrease in enzyme activity.

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P30-2

Direct recovery of intracellular ectoine from *Halomonas salina* cells hydrolysate

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Ectoine is a compatible cell protectant produced by most of the halophilic and halotolerant microorganisms. Ectoine is a revolutionizing substance which can be applied for skin protection as potent moisturizer and anti-ultraviolet sunscreen product. The arising demand of ectoine in cosmetic, pharmaceutical, medicinal and biotechnology fields has urged the search of cheaper sources and production practices of ectoine. In this study, aqueous biphasic system (ABS) composed of poly(propylene) glycol (PPG) 425 and salt was deployed for the recovery of ectoine from *Halomonas salina* cells. Different types of salts (phosphate, citrate, sulfate) were evaluated for their effect on the partitioning behavior of ectoine in the ABS. The preferential partition of ectoine to the salt-rich bottom phase was designed due to ectoine's hydrophilic property. Besides, the effects of salt types, concentrations of PPG 425 and salts, crude loading, pH, addition of neutral salts and concentration of potassium chloride (KCl) on the recovery of ectoine were investigated. Optimum recovery of intracellular ectoine was obtained from PPG 425/sulfate ABS with system composition of 30% (w/w) PPG 425 and 20% (w/w) sulfate, 15% (w/w) crude loading and 1.5% (w/w) of potassium chloride at pH 6.5. High yield of 94.7% for the recovery of intracellular ectoine from *H. salina* cells were achieved with enrichment factor of 1.7 and purity of 87.03% by using PPG425/sulfate ABS.

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P31-1

Synergistic effect of biomimetic enzyme complexes with various enzymes for advanced biorefinery by microbial whole-cell biocatalyst

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In the practice of converting biomass into valuable biomaterials, the critical step is the decomposition process to give fermentable monomeric sugars. Thus, the designed microbes based on enzyme complexes are a key biological technology for biorefinery. For utilizing of polysaccharides by simultaneous saccharification and fermentation, a recombinant scaffolding protein from *Clostridium cellulovorans* and chimeric hydrolysis enzymes were assembled as complex system. The utilization of scaffolds for enzyme immobilization involves advanced bionanotechnology applications in biorefinery fields, which can be achieved by optimizing the function of various enzymes. The assembly of minicellulosomes by *Saccharomyces cerevisiae* and *Corynebacterium glutamicum* increased the activity against various lignocellulosic materials by approximately 3-fold compared with control. Also, red algae-degrading complexes increased the activity against the marine biomass substrate by approximately 2-fold. Finally, carbon monoxide (CO) was successfully converted by functional complexes containing carbon monoxide dehydrogenase and carbon monoxide sensing heme protein with enhanced CO binding affinity. An enzyme complex was anchored on the cell surface of CO_2-utilizing *Ralstonia eutropha* and successfully showed 3.3-fold increased conversion efficiency. Intelligent application of various scaffolds to couple with nanoscale engineering tools and metabolic engineering technology may offer particular benefits. The development of multi-functional protein complexes for use as tools in whole-cell biocatalyst systems has drawn considerable attention as an attractive strategy for bioprocess applications.

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P32-1

Towards a sustainable production of biologically active building blocks from dye-rich wastewaters

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Looking beyond the traditional economy of "take, make and dispose" we established a multi-step enzymatic system for an eco-friendly biological treatment of dye-rich wastewaters simultaneously producing commercially interesting compounds. The dyeing process is responsible for the release into the environment of about 2.8×10^5 tons of dyes and dyestuff each year. The major component of these wastewaters are azo (-N=N-) dyes, which are recalcitrant and hazardous molecules. Our aim is to shift this paradigm through the implementation of circular economy policy – recovery, reuse, recycling and remanufacturing of products. An azoreductase isolated from *Pseudomonas putida* MET94 (PpAzoR) with high efficiency in decolourising several structurally different azo dyes was used [1] producing aromatic amines. In a step-wise manner, a laccase from *Bacillus subtilis* (CotA-laccase) previously shown to be active in homo- and heteromolecular coupling of aromatic amines was added to the reaction mixtures [2]. These reactions led to the production of important precursors for biologically active compounds like phenazines, phenoxazinones and quinones. In a first stage, purified enzymes were used sequentially to treat 5 structurally different dyes. Then, in a second stage, to increase process sustainability, removing high costs associated to the use of purified enzymes and of co-factors, a whole cell system was established. Free and immobilized whole-cell bioprocesses of recombinant *Escherichia coli* cells overproducing PpAzoR and CotA-laccase [3] were optimized. Finally, the immobilized biocatalytic system was used in repeated cycles, demonstrating its further sustainability and robustness.

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P32-2

Relation between CH_4 and CO_2 conversion and the production of bioproducts by consortium (algae and bacteria)

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One of the major sources of natural gas production occurs in the oil extraction process where large volumes of natural gas are released. The composition of natural gas can vary widely, but methane (CH_4) is the main component (80–95%), also presenting carbon dioxide (CO_2) contents. However, the release of gases (CH_4 and CO_2, mainly) may be considered one of the main causes of global warming and climate change. Because of that, the need arises to transform natural gas into other products, aiming at mitigating and generating products with higher value. There are some studies focusing on the microbial sequestration of CO_2 and CH_4, but there is not on the microbial conversion of both gases into bioproducts. A microbial consortium involving algae and bacteria can be used. Together, these microorganisms could rapidly metabolize high concentrations of CO_2 and CH_4, to become them into valuable bioproduct. Consortium (algae and bacteria) studied was isolated from mangrover, and it was able to survive in 70% CH_4 and 30% of CO_2. Only CO_2 was also tested and the consortium grew and consumed up to 50% of CO_2. Lipids compositions were studied to see the difference in each growth condition.

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P32-3

The effect of temperature and ammonia on species-dependent coral health status: A comparative case study of *Acropora* sp. and *Porites* sp.

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This research aimed to study the effects of temperature and ammonia on the health status of *Acropora* sp. and *Porites* sp. at Sichang Island, Thailand by using acute toxicity testing (50% lethal concentration: LC50). The acute effects of temperature and ammonia on the above corals were monitored at 24 h and 48 h. The experiments were carried out in triplicate at temperatures of 30 °C and 33 °C. The concentrations of ammonia were varied at 0, 0.05, 0.07 and 0.1 mg N/L, respectively. The active polyp percentages of *Acropora* sp. were analyzed with comparison to the health status percentages. According to the findings at temperatures of 30 °C (at 24 h and 48 h) and 33 °C (at 24 h), the acute toxicity of coral bleaching (LC50) could not be investigated because the effects were insufficient to cause a decline in coral health or the mortality percentages was not below 50%. On the other hand, at a temperature of 33 °C at 48 h, the acute toxicity of coral bleaching (LC50) could be evaluated. The results strongly indicate that the mortality percentages exceeded 50%. The results were confirmed by Zooxanthellae density in seawater. Therefore, the LC50 at 48 h in this study of *Acropora* sp. and *Porites* sp. were equal to 0.043 and 0.054 mg N/L, respectively.

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P32-4

Withdrawn

P32-5

Development of ageoinformatic utility in landfill biotechnologies and sanitary drawings for protecting public health's spaces from associated risks

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This study analyses different waste management techniques in landfill biotechnology influencing on biogas emissions, leachate emissions, organic hazardous toxic acids and landfill biomass biodegradation stages. Moreover, it examines the significance of phytobioremediation techniques in particular landfill heavy metals' concentrations minimisation in time. The biodegradation of waste pretreatment techniques in landfill biotechnology is evaluated based on different biomass waste inputs [3–5].

Different biomass substrates are evaluated based on results from Mid Auchencarroch experimental landfill investigating different cells [1,2]. The variations of the examining emissions are analysed in order to develop an efficient monitoring system in landfill biotechnologies for the right monitoring of particular bio-

process stages; supported constructions and decision making in taking measures for soil protection in agricultural activities and food safety for public health protection and supporting associative activities in community health centres [6–8].

Useful results are presented for monitoring systems in landfill biotechnologies based on a geoinformatic risk assessment utility that develops useful evaluations and sanitary drawings in efficient designs that support minimisation in associated public health's risks at outdoors and indoors pollution spaces; agricultural soil protection, water resources, environmental resources' protection and food protection.

Bioeconomy's principles and demographic scenarios are evaluated for public health protection.

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P32-6

Hydrogels of chitosan as bioadsorbents of Cr (VI) in aqueous systems

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The increase of the contamination in aquifers by heavy metals in the central zone of Mexico (Bajio region) has brought impacts on the environment and on public health. Several studies have shown that the concentrations of Cr (VI) in the zone exceed the maximum allowable limit (0.05 ppm) by the Mexican regulations NMX-AA-044-SCFI-2014. In this context, several investigations have focused on finding an ideal bio-adsorbent for the removal of this metal. The aim of the present work was to synthesize hydrogels based on chitosan with glutaraldehyde to evaluate the adsorption capacity of Cr (VI) in aqueous solutions by means of batch experiments. Five different concentrations of Cr (VI) were evaluated: 20, 30, 50, 80 and 100 ppm, using 6 g of the hydrogel. The parameter evaluated was the adsorption percentage after 90 min. The amount of Cr (VI) adsorbed was quantified every 10 min. The results showed that after 20 min the percentages of adsorption were greater than 50% with a maximum percentage of adsorption of 97.55%. These results showed that hydrogels based on chitosan with glutaraldehyde are efficient in the removal of Cr (VI) at concentrations between 20 and 100 ppm. This shows its potential application in the treatment of effluents contaminated with Cr (VI) in the Bajio region.

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P32-7

Efficient production of reuterin from glycerol by *Lactobacillus reuteri*

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Reuterin (3-HPA, 3-hydroxypropionaldehyde) is a precursor to 1,3-propanediol (1,3-PDO), which is a potential valuable chemical and new broad-spectrum anti-microbial substance. The aim of the present work was to optimize reuterin production by *Lactobacilus reuteri* OH0335 using a two-step process from glycerol. The first step was the *L. reuteri* cells growth in optimal conditions and the second step was harvesting the cells by centrifugation and suspended in glycerol solution for reuterin production under different conditions. We investigated the influence of cell harvest time, glycerol concentration, biomass concentration, pH, temperature, the molar ratio of glucose/glycerol, metal ions (Mg^{2+}, Co^{2+}) and VB12 on the production of reuterin. At the optimized conditions, reuterin concentration of 300 mM was obtained after 1 h of incubation at 30 °C in 500 mM glycerol for an initial resting cell OD100. The reuterin conversion yield was 0.6 M reuterin/M glycerol.

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P32-8

Archaeal community structure in the anaerobic sludge digesters of a full-scale municipal wastewater treatment plant in Dubai, United Arab Emirates

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The production of methane in the anaerobic digesters of wastewater treatment plants is carried out by diverse microbial communities, which remain poorly understood, especially the Archaea. This study investigates the methanogenic archaeal communities in three digesters of municipal wastewater treatment plants using culture independent techniques, i.e. fluorescence in-situ hybridization and quantitative polymerase chain reaction. Multiple probes targeting domain archaea, different orders and families of Archaea were used for the studies. The results show the dominance of the members of the family Methanosaetaceae and Methanosarcinaceae in these digesters. Since members of these two families are aceticlastic methanogens, their dominance suggests that probably the methane in the tested digesters is produced through the conversion of acetate into methane. Interestingly, the population of total archaea increased in the months of November and December with a corresponding increase in the population of

family Methanosarcinceae that are mesophilic. This suggests that the ambient temperature influence the population of Archaea in the digester and probably favors the growth of Methanosarcinaceae. This study is first such report on archaeal microbial communities in anaerobic digesters of a full-scale municipal wastewater treatment plant in United Arab Emirates. Further, studies with an aim to understand and improve the process of anaerobic digestion are highly desirable.

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P32-9

Evaluation of toxicity of HCH isomers and its degradation metabolites on mammalian cell line and zebra fish embryos

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Hexachlorocyclohexane (HCH) is a Persistent Organochlorine Pesticide (POPs) which is banned but continues to be illegally used in many developing countries like India. All HCH isomers are recalcitrant, potentially toxic and some of them are considered carcinogenic and potent endocrine disrupters. Our experiments have shown that HCH isomers produce several metabolites during their metabolic fate in the environment. We investigated the effects of different HCH isomers and the degradation metabolites on human cell line VERO and HEK-239 for their toxicity/carcinogenicity. Experiments were conducted for 24 h and for 4 days using HCH concentrations ranging from 0 to 100 μM. The preliminary indications point towards toxicity of α-, β-, γ- and δ-HCH isomers and carcinogenic effects of β-HCH within these concentrations. Further in vivo cytotoxicity study of HCH isomers and their metabolites were assessed in embryonic zebra fish model. Determination of cytotoxicity of the isomers and its metabolites was studied by uptake analysis of the isomers and intracellular ROS determination with the help of flow cytometry.

Also the preliminary indications pointed towards toxicity of all the tested HCH isomers and their metabolites (β-, δ-, B2 and D2) invariably at 40 μM conc. Complete cell death, ROS production and apoptosis were evidently observed at 36 h. However deformities in the cells could be seen even after at 2 h of treatment.

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P32-10

Potential use of biofumigant from *Streptomyces mycarofaciens* for protection of maize seed contaminated by aflatoxin-producing fungi

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Effect of volatile compounds of *Streptomyces mycarofaciens* to protect maize seed contamination by the aflatoxin-producing fungi was investigated. Among many pathogenic fungal strains, the volatile compounds from 12-day old culture of *S. mycarofaciens* on wheat seed exhibited the most pronounced suppression on *Aspergillus parasiticus* and *A. flavus*. The volatiles were identified by using GC–MS and found to contain more than 30 compounds with 2-methylisoborneol was the major component. The wheat seed culture of *S. mycarofaciens* could totally inhibited the two aflatoxin-producing fungi tested in vitro and on maize seed. The main action of volatile compounds from *S. mycarofaciens* was on the conidia germination of the two aflatoxigenic fungi. Therefore, *S. mycarofaciens* has high potential as source of biofumigant to control aflatoxin-producing fungi.

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P32-11

Recovery of diseased rice seeds by biopriming with herbicide-resistant endophytic bacteria of rice plants

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Post-harvest disease of rice seeds causes severe loss of seed quality and fertility. Here, we aim at evaluating biotechnological benefits of herbicide-resistant endophytic bacteria to recover the health of diseased rice seeds by biopriming technique. Five among all isolates of herbicide-resistant endophytic bacteria (isolated from tissues of rice plants grown in the fields applied with different herbicides) gave the best antifungal potential against a set of phytopathogenic fungi isolated from diseased rice seeds. These endophytic bacteria were strains of the genus *Bacillus* classified by phylogenetic analysis of their 16S RNA gene sequences. Biopriming technique comprised of soaking infected rice seeds in the cell suspension of the best herbicide-resistant endophytic bacteria at different time series, followed by air drying till the moisture dropped <12% and keeping at 4 °C for 6 months before tests. Non-priming and priming of rice seeds with sterile distilled water served as controls. We found that biopriming could enhance the germination rate of diseased rice seeds. Full-scale pot tests since germination to production phases after biopriming also optimized growth of rice seedlings lead to the higher yield of rice compared to the controls. With these findings, our biopriming protocol would be a promising green technology for recovering and maintaining the quality and fertility of diseased seeds for further use in organic agriculture with the acceptable crop yield.

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P32-12

Withdrawn

P33-1

The production of methacrylic acid in *Escherichia coli*

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Methacrylic acid is used to produce polymethylmethacrylate, a transparent polymer, used to manufacture Perspex and a wide range of other materials. We engineered *Escherichia coli* to produce methacrylic acid, by using a thioesterase to hydrolyse methacrylyl-CoA, a naturally-occurring intermediate of valine metabolism. Candidate thioesterases were identified by screening databases for enzymes acting on structural analogues of methacrylyl-CoA. The candidates were expressed in *E. coli* and tested *in vitro* for methacrylyl-CoA hydrolysis. 4-Hydroxybenzoyl-CoA thioesterase (4-HBT) from *Arthrobacter* sp. Strain SU was found to hydrolyse methacrylyl-CoA selectively, with minimal hydrolysis of the precursor, isobutyryl-CoA.

Production of methacrylic acid from glucose was demonstrated by co-expressing the genes encoding branched chain keto acid dehydrogenase (BCKD) from *Pseudomonas putida* KT2440, an acyl-CoA oxidase, ACX4 from *Arabidopsis thaliana*, and 4HBT. The methacrylic acid titre was 167 ± 7.3 μM MAA in shake flasks, from 5 g L^{-1} glucose. Surprisingly, production was not improved by over-expressing *alsS* from *B. subtilis* 168, *ilvC* from *E. coli* MG1655 and *ilvD* from *E. coli* MG1655 from a separate plasmid, even though this combination of genes is known to improve flux from pyru-vate to 2-ketoisovalerate in *E. coli*. Bio-based isobutyric acid can also be produced from sustainable feedstocks, so we also expressed acyl-CoA synthetase (*AcsA*) from *Pseudomonas chlororaphis* B23, to activate isobutyric acid to isobutyryl-CoA, alongside ACX4 and 4-HBT. In this way, 367 μ M methacrylic acid was produced from 5 mM isobutyric acid in shake flasks. Current work is focussed on yield improvements by enhancing flux to product and overcoming product inhibition.

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P33-2

Improvement of vision, cognitive and functional performance through erinacine A-enriched *Hericium erinaceus* mycelia in patients with mild Alzheimer's disease

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Hericium erinaceus is an edible mushroom that contains active compounds called hericenones and erinacines, which can only be extracted from the fruit body and the cultured mycelium, respectively. When *H. erinaceus* mycelia is enriched with erinacine A and included in daily meals, preclinical studies showed improvements in neurodegenerative diseases such as ischemic stroke, Parkinson's disease, and Alzheimer's disease. Moreover, it could decrease β-amyloid plaque burden and promote survival of newly born neurons in the hippocampal dentate gyrus of APP/PS1 transgenic mice. Higher scores of nest building behavior were also detected in the mycelia treated group compared to the untreated group. Based on these encouraging results in animal studies, the aim of this study is to investigate erinacine A enriched-*H. erinaceus* mycelia's effect in patients with mild Alzheimer's disease. In total, 36 participants having mild Alzheimer's disease (Mini-Mental State Examination (MMSE) score, <20) were recruited. For assessing the severity of the Alzheimer's disease, five questionnaires (Neuropsychiatry Inventory (NPI), Instrumental Activities of Daily Living (IADL), Clinical Dementia Rating (CDR), Cognitive Ability Screening Inventory (CASI), and MMSE) were used. Results showed that significant improvements in assessment scores after 42 weeks were observed for the NPI, CDR, and MMSE scores. Moreover, mycelia treatment significantly increased serum brain derived neurotrophic factor while significantly decreased β-amyloid and apolipoprotein E4 levels after treatment. Taken together, these results indicated that erinacine A enriched-*Hericium erinaceus* mycelia may help slow the progression of the disease and improve the quality of life in patients with mild Alzheimer's disease.

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P33-3

Prevention of allergic inflammation by oral administration of bromelain in a murine asthma model

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Bromelain is an enzyme mixutre extracted and isolated from the stems of pineapples (*Ananas comosus*). This material has high proteolytic activity, and it has also been acknowledged as a natural anti-inflammatory agent. Many researches have revealed that inflammation induces and advances many diseases, such as allergy, cancer, diabetes, cardiovascular diseases, and even neurodegenerative disorders, thus the demand of the natural anti-inflammatory product keeps increasing in the nutraceuticals market.

Chappion Biotechnology and SunTivas have developed a nutraceutical product containing Bromelain obtained from the pineapple stems grown in Taiwan, and a murine asthma model was used to demonstrate its anti-inflammatory effect. The results revealed that the oral administration of Bromelain significantly reduced the breathing resistance in the sensitized mice. The blood analysis showed that Bromelain reduced the levels of IgE, OVA-IgE, TNF-α and IL-4, whereas the levels of IgA and OVA-IgG1 were not affected. This animal trial discovered that Bromelain relieved the symptoms of the allergic asthma by inhibiting the Th2 immune responses. As a result, Bomelain is indeed a natural and effective product for preventing inflammation.

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P33-4

Scaling up lycopene production by *Blakeslea trispora* in diffused bubble column reactor

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The present study aimed to examine the feasibility of scale up from the surface-aerated shake flasks to dispersed-bubble aer-

ated bench scale (BCR) (scale up ratio of 1:20) on the production of lycopene by *B. trispora*. To achieve this goal, Response Surface Methodology and Central Composite Statistical Design were applied for the systematic process optimization in BCR, combining the action of the stimulants, namely crude soybean oil (CSO) and 2-methyl-imidazole addition level, and airflow rate. A reversed-phase high-performance liquid chromatography procedure was used to monitor the lycopene content and the selectivity of the bioprocess in fungal cells. Considering that the initial volumetric mass transfer coefficient (k_La) is a key response that influences the success of the scale-up process, the quantitative relationship of initial k_La with the independent factors was also examined. The optimized levels of factors were 110.5 g/L (CSO), 2.3 vvm (airflow rate) and 29.5 mg/L (2-methyl-imidazole). At these optimum conditions, maximum lycopene yield (256 mg/L) was comparable or even higher to those reported in shake flasks. 2-Methyl imidazole use at levels significantly lower than those reported for other inhibitors in the literature was successful in terms of process selectivity. CSO provides economic benefits to the process through its ability to stimulate lycopene synthesis, as an inexpensive carbon source and oxygen vector at the same time. Current findings, along with the advantages of BCR, suggest the favorability of using this system for lycopene production by *B. trispora*.

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P33-5

Experimental and in silico development of molass based lactic acid fermentation technology

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Lactic acid is a special renewable and sustainable platform forming chemical having wide applications of both itself and of its derivatives in several industries including pharma, feed&food, cosmetic and polymer industries. Our research group develops fermentation based lactic acid manufacturing technologies over a decade. Generally we study and develop this process from different point of views according to the demand of our different industrial partners. We already studied mesophilic and thermotolerant lactic acid production [1], direct lactic acid fermentation from starch [2], wheat [3], and sweet sorghum [4] based fermentations. Recently we have to adapt our most economic process with some modifications to sugar cane molasses as raw material [5] as well as to an existing facility of our partner. In this report the techno-economic flow sheeting based in silico modelling of this process is studied and presented, and different scenarios are evaluated. This calculation considers our recent investigation of both upstream and downstream processes, like the solutions of challenging molasses application and a precipitation based product recovery.

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P33-6

The emerging organism engineering industry

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There is an emerging demand for sourcing plant-derived extracts such as nutraceuticals, flavors, fragrances, sweeteners, etc. and animal derived proteins from engineered microbes. While recent advances in synthetic biology and metabolic engineering provide feasible approaches to engineering such organisms, commercial success for developing these “cultured” ingredients and “animal proteins” present specific challenges. Unlike biofuels, where efforts can be focused on one particular molecule given the enormous market size, cultured ingredients and cultured animal proteins require developing different organism lines in a rapid and low-cost fashion. This requires a scalable solution for biomanufacturing of organisms, which is provided by our state of the art foundry that continues to grow. Engineering microbial organisms for the commercial production of any desired molecule requires repeated iterations of the design-build-test engineering cycle in order to optimize the metabolic flux of the production host. I will describe how organism development at Ginkgo Bioworks leverages our foundry to accelerate the design-build-test cycle using specific examples. In particular, I will highlight the value of combining computer-aided engineering software tools, cheap gene synthesis and high resolution-accurate mass LCMS to develop engineered microbes. Finally, I’ll touch on how our improvements in manufacturing organisms lead to opportunities outside of cultured ingredients and proteins.

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P33-7

Yeast cell production from vegetable oil

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Microbial utilization of lipid will be significant in supplying proper nutrition for animal and fish consumption from safe and cheap raw material, and in resolving the treatment of waste lipid. We have attempted to produce yeast cell from vegetable oil and obtained some yeast strains which grew on vegetable oil as sole carbon source from natural sources. *Meyerozyma guilliermondii* TY-89 capable of assimilating both commercial vegetable oil (the mixture of soybean oil and rapeseed oil) and waste vegetable oil effectively was found among them. We carried out study on cultural condition of this strain on commercial vegetable oil. The medium contained commercial vegetable oil as sole carbon source, ammonium sulfate as nitrogen source, and some minerals. One hundred millilitres of the medium in a 500 ml Erlenmeyer flask was inoculated with 4 ml of precultured broth and incubated at 30 °C. When cultivating this strain in Erlenmeyer flasks with buffles on a rotary shaker (120 rpm), cell growth was better than that in Erlenmeyer flasks without buffles. The buffle can change large droplets of vegetable oil to small ones. It seems that small droplets of vegetable oil are assimilated more rapidly than large ones. The ammonium group was effective as nitrogen source, nitrogen of nitrate group was hard to assimilate. This strain can below pH 2.0 and addition of ammonia water was effective cell growth. These results

suggest that this strain is suitable for making yeast cell from vegetable oil.

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P33-8

Bio-based cementation using urease-producing bacteria: Sand solidification and kinetics

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Calcium carbonate ($CaCO_3$) is a well-known cementing material and also plays an important role in biominerals. The formation of artificial $CaCO_3$ as a cementing material via microbial processes is called microbially-induced carbonate precipitation and would be an ecofriendly method in the field of geotechnical and civil engineering. Urease producing bacteria are promising candidates for MICP, where urea is hydrolyzed to carbonate and ammonia, followed by $CaCO_3$ formation in the presence of calcium ion. The precipitated $CaCO_3$ acts as a binding material, which binds sand grains together at the particle-particle interfaces, leading to solidification of sand or soil.

We isolated urease-producing bacteria, *Pararhodobacter* sp., which exhibits high solidification ability toward a variety of sand and soil. In the present study, we examined the solidification of sand using the bacteria in column system, and a kinetic study of $CaCO_3$ precipitation associated with urea hydrolysis. Kinetics study revealed that $CaCO_3$ precipitation was dominated by urea hydrolysis rate which can be estimated by Michaelis–Menten model. We also studied the effect of poly-lysine addition on $CaCO_3$ precipitation by the bacteria. In the presence of poly-lysine, morphology changed drastically from rhombohedral crystals to twin sphere-shaped crystals. Furthermore, by adding poly-lysine, strongly cemented sand specimen could be obtained with the higher strength than the conventional method.

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P33-9

Cultural characteristics *Meyerozyma guilliermondii* TY-89 on lard

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Lard is used in many restaurants and food industry companies in Japan. However, waste lard does not seem to be reused effectively. Therefore, we have attempted to produce yeast cell from lard for food or feed. Some yeast strains which grew on commercial vegetable oil (the mixture of soybean oil and rapeseed oil) or olive oil were obtained from some flower's petals. A yeast strain capable of assimilating lard effectively was found among them, and named *Meyerozyma guilliermondii* TY-89.

We carried out studies on cultural conditions of this strain. The medium contained commercial lard as the sole carbon source, ammonium sulfate as nitrogen source, and some minerals. One hundred millilitres of the medium in a 500 ml Erlenmeyer flask with buffles which can change large lump of lard to small ones was inoculated with 4 ml of precultured broth and incubated at 30 °C on a rotary shaker (120 rpm). The cell growth was evaluated using dry cell weight.

The cell growth in Erlenmeyer flask with buffles was better than that without buffles. On the other hand, the cell growth on lard was not better than that on vegetable oil under the same cultivation condition. The major fatty acids in lard ware oleic, palmitic, stearic, and linoleic. This strain grew with oleic acid as the sole carbon source at a rate similar to that for growth on linoleic acid. But this strain grew with palmitic acid slowly and with stearic acid little, respectively.

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P33-10

From meat by-products to peptones by enzymatic hydrolysis. Application in microbiological growth media

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Premium and low cost nitrogen sources for microbial growth were prepared from meat by-products. Peptones were obtained using Alcalase and Neutrase through the optimisation of various parameters such as time, temperature, pH and E/S ratio (weight/weight of protein). The parameters were studied by single factor experiments and response surface methodology thought Central Composite Design (CCD). Under optimal conditions the degree of hydrolysis are close to 25% for Alcalase and 9% for Neutrase. The nitrogen content of peptones prepared with optimized conditions range 10–12%, similar to commercial peptones. SDS-PAGE analysis was used to determine molecular weight distribution, showing that the peptones obtained were smaller than 15 kDa. The performance of the meat peptones produced with Alcalase was compared with commercial peptones in a biotransformation process. Peptones were used as nitrogen source for the growth medium of different fungi strains. 5-Hydromethylfurfural (HMF) was added and its biotransformation to HMF derivatives was determined observing that the meat hydrolysates prepared with Alcalase can be an alternative to currently available commercial nitrogen sources. A bio-economic study is in progress to assess the viability of this process at industrial scale.

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P33-11

Identification of anti-aging natural products from southwestern Indian Ocean marine sponges and their microbial symbionts

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Aging is a complex molecular process representing one of the main risk factors for major human pathologies such as Alzheimer's disease or cancers. As the population of developed countries is aging, the prevalence of a variety of age-related diseases is increasing. In this regard, marine natural products (MNPs) represent an extraordinary reservoir of structurally diverse bioactive metabolites. Among them, promising novel compounds may offer anti-aging properties to pharmaceutical and cosmeceutical industries, in order to counteract this major healthcare challenge.

To overcome existing bottlenecks in industrial exploitation of MNPs, the H2020 European project TASCMAR explores marine invertebrates and symbionts from under-investigated marine biodiversity hotspots and develops innovative approaches for

the extraction of these organisms. Among marine organisms, sponges are the most prolific source of newly discovered bioactive molecules. Furthermore, the role of their associated microorganisms in this production is now more and more explored. As partner of TASCMAR, the LCSNSA possesses in its collection 12 marine sponges out of 47 collected in Mayotte, presenting inhibitory activities against different molecular targets [elastase/tyrosinase (skin aging), Fyn kinase (Alzheimer's disease), proteasome/CDK-7 (cancer)]. Amongst these organisms, *Scopalina hapalia* was selected for a detailed study of its chemical composition and microbiome. To date, we identified in its microbiome 26 bacterial strains clustered in 5 genera (*Bacillus*, *Jishengella*, *Micromonospora*, *Rhodoccocus*, *Salinispora*) and two unidentified isolates related to the family Thermoactinomycetaceae. Moreover, 124 crude extracts from 31 bacteria and filamentous fungi associated to *Scopalina hapalia* have been evaluated for their anti-aging potentialities.

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P33-12

Application of lignin as a natural active ingredient in sunscreens

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Lignin, the second most abundant renewable resource in the world, is obtained in large quantities as a by-product of pulping and biorefinery processes. Lignin has been treated as waste and used in low-value applications although it possesses attractive functions such as antioxidant, antimicrobial, and ultraviolet (UV) protecting activities. Long-time exposure to the sun's UV radiation is harmful and causes various skin problems. Sunscreens are commonly used to protect skin from UV rays. Active ingredients in sunscreens are often synthetic chemicals, and long-term use of such chemicals may cause unexpected side effects on skin. Therefore, natural sun blockers have been attracting considerable attention in recent times. Even though lignin is a natural UV blocker, its unfavourable dark colour hinders its high value-added applications in sunscreens and cosmetics. In this work, we isolate lignin under mild conditions in order to prevent darkening occurring during delignification of lignocellulosic biomass, and apply the resultant lignin as a natural sunscreen agent for the first time. Lignin (ML) isolated from grass and from wood are compared with organosolv lignin (OL), which showed the best sunscreen performance, in colour and UV protection. The MLs were light in colour unlike conventional lignins extracted under harsh conditions. UV absorption of light-coloured ML was revealed to be as high as dark-coloured OL. The MLs also showed synergistic effects when blended with a commercial sunscreen.

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P33-13

Enhanced n-butanol tolerance of *E. coli* via adaptive evolution

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Synthetic biology allows the engineering of microorganisms to produce a wide variety of industrially relevant chemicals in a green and sustainable manner. However, production levels are usually limited by the inherent toxicity of the bioproduct to the production host. Such is the case with the bioproduction of n-butanol, when using either naturally producing *Clostridium* spp. or alternative production hosts. *Escherichia coli*, one of the most studied hosts for n-butanol production, can tolerate n-butanol at concentrations up to 0.75–0.80% v/v (6 g/L) before growth is completely inhibited. Therefore, we aimed to improve the n-butanol tolerance of *E. coli* BW25113 by adaptive evolution. The evolution process was achieved by growing *E. coli* in minimal medium containing exogenous n-butanol and subculturing every 24–48 h. The n-butanol concentration was gradually increased from 0.5% (v/v) to 1.15% (v/v), and the population was evolved for a total of 100–150 subcultures. The evolved population could grow at butanol concentrations up to 1.40% (v/v) (11 g/L), an improvement of 1.75-fold compared with the parental strain. Future work will focus on understanding the mechanism of tolerance using Omics analysis, and rational engineering to further improve n-butanol tolerance.

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P33-14

Development of technology to increase *Haematococcus* sp. biomass by inducing turbulence in tubular photobioreactor

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Microalgae are single-celled plants that produce a variety of secondary metabolites through photosynthesis. In order to industrially cultivate these microalgae, it is necessary to develop a photobioreactor system capable of mass culture. Tubular photo bioreactors are representative microalgae mass culture systems. However, such a culture system has microalgae adherence inside the tube during cultivation, and when the density of the cells is increased, the light is restricted between the cells. One of the causes of this phenomenon is the laminar flow inside the tube. Therefore, in this study, we tried to increase the growth of microalgae by inducing turbulence by applying various mixer inside the tube. Also, In order to change the flow of the culture fluid flowing in a certain direction, the pump was installed at both the beginning and the end of the tube so that the fluid inside the tube could flow in the opposite direction. As a result, the unidirectional flow of fluid can be periodically reversed, and the adhesion of microalgae can be suppressed. Based on the above results, *Haematococcus* was cultivated in the improved photobioreactor, and the biomass was about 1.5 times higher than before the improvement. In addition, it was confirmed that even after continuous operation for 4 weeks or more, microalgae were not adhered to the inside of the tube, and thus it can be effectively produced

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P33-15

Industrial effluent treatment in aspect of biocatalyst deactivation kinetics

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Conventional effluent treatment technologies are unable to fully remove all contaminant substances from water. Standard biological treatments can deplete, partially or even completely, several organic substances. However, conventional biological treatments are unable to fully remove all substances from water. In biotechnology, a biological material is used to realize a production in

commercial scale. In this case, biocatalysts can be employed to achieve a selective removal of compounds in effluent treatment. Lately, the increasing interest to these biotechnological processes, stability and activity of biocatalyst is becoming more important in aspect of product quality and economy. So, the description of the deactivation of biocatalysts is very important from industrial applications viewpoint.

Generally, the first-order kinetics is most frequently accepted for deactivation kinetics model. However, in many cases, experimental data do not correspond to simple first-order kinetics, especially when dealing with immobilized biocatalysts. The reasons for deviation from simple first order may be various. For example, it may be an intrinsic heterogeneity of biocatalysts, the presence of different cells catalyzing the same reaction, the complexity of the mechanism of deactivation, etc.

In this current study, dairy industry effluent biological treatment and inactivation kinetics of biocatalysts were investigated. Immobilized cells were used as biocatalysts. The fixed bed reactor was constructed for this study. From the evaluation of results, it was observed that there are two different deactivation regions, activity and stability, with respect to time. Two stages deactivation model was described by equation as follow.

$$A_t = a\,\exp(-k_a t) + b\,\exp(-k_b t)$$

The results were evaluated according to this model and the kinetic constants of biocatalyst were determined.

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P33-16

Furan fatty acid, EODA: A new candidate for antibacterial agent against multidrug-resistant *Staphylococcus aureus*

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Structural modification of natural lipids by biocatalysis can change their properties or even create novel functionalities. Hydroxy fatty acid is one of the modified fatty acids which can be produced from natural vegetable oils by the microbial bioconversion. Recently 7,10-dihydroxy-8(E)-octadecenoic acid (DOD) was produced from olive oil by a bacterial strain Pseudomonas aeruginosa PR3. Further study confirmed that DOD contained strong antimicrobial activities against broad range of microorganisms. In this study we tried to modify DOD molecules by physical treatment to create new functionality or to enhance the antimicrobial activity of DOD. After the harsh heat-treatment, a novel furan fatty acid (EODA) was produced from DOD. We confirmed that EODA presented strong antibacterial activity against multidrug-resistant Staphylococcus aureus and also EODA showed a recuperative effect of the beta-lactam antibiotics activity against methicillin-resistant *Staphylococcus aureus*.

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P33-17

Geobacillus sp. 95 as a source of enzymes with special characteristics: Characterization of thermostable lipases, esterases, ureases and nitrate reductases

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Geobacillus bacteria are widely distributed and readily isolated from natural and man-made thermophilic biotopes. As with many thermophiles, considerable interest in potential industrial application of these bacteria and their thermostable enzymes exists. In previous works we have shown that *Geobacillus* sp. 95 strain produced lipolytic enzymes (GD-95 lipase and GDEst-95 esterase) with attractive characteristics for industrial application. It has been shown that a chimeric variant composed of these two enzymes also demonstrated high potential for the lipid bio-industry.

In this work we analysed the potential of several *Geobacillus* spp. strains to produce ureases, which can be applied in biocement production and microbial calcium carbonate precipitation. Screening by classical microbial methods using selective urea-broth medium and molecular detection by α subunit-specific primers allowed the selection of *Geobacillus* sp. 95 as a perspective urease-producing strain. Another important industrial and pharmaceutical application of microorganisms is production of silver nanoparticles. The most significant enzyme in bacterial-based silver nanoparticle synthesis is nitrate reductase. In this study it was shown that *Geobacillus* sp. 95 strain could successfully perform synthesis of silver nanoparticles and possessed high nitrate reductase activity. Based on the obtained experimental results, *Geobacillus* sp. 95 strain and thermostable enzymes produced by this strain show high perspectives for further application in industry.

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P34-1

Optimizing cell density of *Pichia pastoris* for production of recombinant hepatitis B surface antigen via employing short-period continuous operation

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In biopharmaceutical industry, high cell density fed-batch cultivation is the main approach for production of recombinant protein with high yields in methylotrophic yeast *Pichia pastoris* (*P. pastoris*). In current study, in addition to conventional fed-batch process, we employed short-period chemostat fermentation to control and manipulate the cell density of *P. pastoris* in the pilot-scale fermenter easily. For this purpose, after reaching the maximum broth volume of 5 L in the fed-batch process, the operation mode was changed to chemostat fermentation, under the DO-stat condition, with the dilution rate of 0.009, and the process continued until reaching the steady-state point. To alter the cell density in the chemostat fermentation stage, the inflow of methanol, as a limiting nutrient, was adjusted to various values between 31 ml/h and 42 ml/h. In each selected point, the cell density, methanol consumption rate and amount of both total protein and recombinant hepati-

tis B surface antigen (rHBsAg) were measured. According to the results, the optimal methanol inflow based on yield, productivity and ease of process control was 40 ml/h. In this flow rate the cell density increased from 363 mg/ml WCW in the fed-batch stage to 450 mg/ml WCW. In the selected operation condition, the titer, volumetric and specific productivity were 188.8 mg/l, 1.7 mg/l/h and 0.00468 mg/g/h, respectively. The obtained cell density is suitable for large-scale fermentation process without creating any major issues such as excessive heat dissipation or very low concentration of dissolved oxygen.

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P34-2

Affinity purification of recombinant proteins using a LysM domain and bacterium like particles

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The lysin motif (LysM) is a ubiquitous motif across kingdoms, which in bacteria allows cell wall degrading enzymes to bind non-covalently to peptidoglycan. This property has been exploited for two decades to design mucosal vaccines consisting of LysM-tagged recombinant proteins anchored to bacterium like particles (BLP) as carriers. Surprisingly, less attention has been paid to apply the LysM motif to protein purification of recombinant proteins. Thus, our goal was to determine if the LysM motif is suitable for recombinant protein purification.

We obtained the BLPs by treating overnight cultures of lactobacilli with acid and heat to get rid of other cell wall components that may interfere with binding. To select the best binding matrix, we generated BLPs from 3 different *Lactobacillus* species: *L. rhamnosus, L. fermentum*, and *L. vaginalis* and checked them by transmission electron microscopy. We constructed a fusion protein consisting of the yellow fluorescent protein Venus fused to a module containing five LysM motifs derived from a *Lactobacillus* sp. strain. The recombinant protein was expressed in *E. coli* Rossetta using standard procedures, and the supernatant containing the fusion protein was incubated with BLPs for binding. We evaluated the effectiveness of binding by fluorescent microscopy and SDS-PAGE. After binding, the complex was washed several times, and the elution of the protein was tested by changing pH, ionic strength and buffer composition. As a conclusion, we demonstrate that the LysM motif can be used as novel tag to purify recombinant proteins by affinity using an economical matrix, obtaining similar yields to the NiNTA system for protein purification.

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P34-3

Purification and characterisation of thermostable catalase AfKatG produced in *E. coli*

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Catalases are widely used in various industrial applications (food industry, pharmaceuticals, biopolymerization, etc.). We have purified and characterized thermostable catalase AfKatG produced in *E. coli* [1] to homogeneous protein quality and activity. We used a simple three steps purification-heat treatment, IMAC nickel column and dialysis against water. We determined pH and temperature optima for both catalase and peroxidase activity and used calorimetric studies of the thermal denaturation to study the melting point of AfKatG. We studied the secondary structure of enzyme by using circular dichroism in far to middle ultraviolet spectrum and we determined its tertiary structure by HPLC.

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Reference

[1] Struhárňanská, et al. New strategies in production of thermophilic catalase-peroxidase AfKatG in *Escherichia coli*. FEBS J 2017, the 42nd FEBS Congress.

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P34-4

Producing new bacteriocins using recombinant microorganisms

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Bacteriocins are antimicrobial peptides traditionally used as food preservatives but with expanding applications to human or animal health. Genetic modifications of these proteins can improve their antimicrobial activity and they have been proposed as a substitute for antibiotics.

The objective of this work was to obtain new engineered bacteriocins with improved antimicrobial activity and technically feasible scale-up of the production and purification process.

First, a new bacteriocin was isolated from *Lactobacillus curvatus* in meat product. The antimicrobial effect of this protein was studied against various pathogens of animals, selected by their prevalence in farms.

Then, we focused on generating custom designed bacteriocins using genetic engineering techniques. A total of 10 genetic modifications were designed and evaluated by protein fusion in the C and N terminal to increase their antimicrobial activity. Additionally, post-translational modifications have been made to improve their stability. For their subsequent production and purification, an affinity tag has been added.

In order to assess the technical feasibility of the biosynthesis process for bacteriocin production, the fermentation conditions were studied and scaled-up, using a stirred tank bioreactor. The

downstream operations were then defined, including the purification through affinity chromatography (AC) combined with different polishing steps using a Fast Protein Liquid Chromatography (FPLC) equipment. Different strategies were applied during the downstream process to identify the best one which obtain high purity and yield with minimum purification steps The samples were analyzed to quantify purity and total protein concentration in each step to show the most promising strategy on industrial scale.

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P34-5

Expression of the ectodomain of the rabies virus glycoprotein G in insect cells for diagnostic purposes in vaccinated llamas

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Rabies is one of the principal zoonosis with worldwide distribution. The disease in llamas produces great economic losses in the productive system. The prevention against rabies virus (RABV) infection is done through vaccination with the inactivated virus. The level of protection can be assessed by determining the titre of neutralizing serum antibodies induced by RABV glycoprotein (RABV-G) in vaccinated individuals, which can be measured using an enzyme-linked immunosorbent assay. The aim of this work is to optimize a process for the production of the RABV-G ectodomain in Sf9 cell. The recombinant baculovirus was constructed by introducing the sequence corresponding to the ectodomain region of RABV-G, under the polyhedrin promoter, fused to the viral signal peptide GP67 at its amino terminus and a 6-histidine tag at its carboxyl terminus. Despite the inefficient secretion of the RABV-G, it was possible to recover it from the cell lysate. Insect cells infected at multiplicity of infection of 0.5 at day 3 expressed 7 mg litre-1. The recombinant RABV-G had a molecular mass of approximately 50 kDa according to SDS-PAGE analysis. PGnase F and Tunicamycin treatment confirmed that the protein is glycosilated. RABV-G was identified by LC-MS and was purified by metal ion affinity chromatography directly from the cell extract with a yield of 99% and a purity of 67%. Purified RABV-G was succesfully used to detect specific antibodies in serum samples derived from rabies-vaccinated llamas. These results indicate that the recombinant RABV-G can be used as an antigen in the development of diagnostic kits.

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P34-6

Production of influenza virus proteins in stably transformed insect cells

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Virus-like particles (VLPs) are composed of one or several recombinant viral surface proteins that spontaneously assemble into particulate structures similar to authentic virus particles. VLPs can be used as a highly effective vaccine that elicits strong immune responses. While the baculovirus-insect cell system has been widely used for the VLP production, it has several inherent limitations including contamination with progeny baculoviruses. Stably transformed insect cells can be used as an attractive alternative to the baculovirus-insect cell system. In the present study, we investigated the production of influenza VLPs in recombinant insect cells. The DNA fragments encoding hemagglutinin (HA) and matrix protein 1 (M1) of an influenza virus A (H1N1) were separately cloned into the plasmid vectors pIHAbla and pIHAneo. The pIHAbla and pIHAneo contained the *Bombyx mori* actin promoter downstream of the *B. mori* nucleopolyhedrovirus (BmNPV) IE-1 transactivator and the BmNPV HR3 enhancer for high-level expression, together with either a blasticidin or a neomycin resistance gene for use as a selectable marker, respectively. After cotransfection with the prepared plasmids, *Trichoplusia ni* BTI-TN-5B1-4 (High Five) cells were incubated with blasticidin and G418, and cells resistant to the antibiotics were obtained. Western blot analysis of a culture supernatant showed that transfected High Five cells secreted HA and M1 molecules. Sucrose density-gradient sedimentation analysis and dynamic light scattering of the culture supernatant suggested that secreted HA and M1 were in a particulate form. These results indicate that recombinant insect cells may offer a promising approach to influenza VLP production.

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P34-7

Withdrawn

P34-8

Designing next generation platforms for recombinant protein production by genome engineering of *Escherichia coli*

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We propose a paradigm shift in our approach to design improved platforms for recombinant protein production, by addressing system-level issues rather than focusing on the individual steps associated with recombinant protein synthesis like transcription, translation, etc. We demonstrate that by controlling and modulating the cellular stress response (CSR), which is responsible for feedback control of protein synthesis, we can generate hyper-producing strains by utilizing genome engineering strategies. For this, transcriptomic profiling of post-induction cultures, expressing different types of proteins, was done to analyze the nature of the stress response & rational genomic modifications were designed to overcome this stress. Potential knockouts were identified which could ameliorate the effect of this stress response. Two model proteins viz. GFP & L-Asparaginase were used to test the modified strains for increased expression. Interestingly, we observed that different sets of modification led to improved GFP (ΔyhbC + ΔelaA) & L-asparaginase production (ΔelaA + ΔcysW). Simultaneously, we identified the top 10 double knock-out strains which were tested for their capabilities to express "difficult-to-express" protein i.e. Rubella E1 protein by tagging it with sfGFP at the C-terminal using a linker peptide for easy online monitoring. Interestingly, the highest increase in Rubella levels was obtained in the same double knock-out ΔelaA + ΔcysW (5.6 fold increase) which gave the highest expression for L-asparaginase. These results indicate that there is a fair degree of commonality in the nature of the CSR generated by the induction of different proteins. We also did transcriptomic profiling of top double knock out strain to demonstrate the ability of former to counter cellular stress more efficiently as compared to the control strain.

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P34-9

Development of bioactivated and functionalized biomaterials by domains of human fibronectin

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Integrin-mediated cell adhesion to biomolecules adsorbed onto biomedical devices regulates their integration and performance. Integrin–fibronectin (FN) interactions have been largely studied, especially in osteoblastic function and bone formation. In this study, we successfully developed a bio-functionalized biomaterial by immobilizing two domains of type III human fibronectin (FNIII9-10) onto a biopolymer (PCL; Polycaprolactone) surface to promote osteoblastic differentiation and implant osseointegration.

In fact, we have developed a new method for producing and purifying the FNIII9-10 in *E. coli*. This strategy allows the real time monitoring of production, purification and immobilization of the recombinant protein by the fusion with a multitags (CMAT-EBI) developed in our laboratory. This system has demonstrated its performance to monitor the expression and the purification of CMAT-FNIII9-10. Using the Multitags as fusion partner, high purity (99%) of recombinant proteins was achieved after two consecutive affinity purification steps. The expression cassette also demonstrated an accurate monitoring capability of the CMATFNIII9-10 adsorption onto the surface of biomaterials. In addition, the bacterial culture was carried out at small scale (100 ml) and transposed to 5L fermentor. Moreover, the extraction of proteins with a homogenizer was optimized by a comparative study with the classical methods (sonication and enzymatic lysis).

An adhesive matrix by combining the properties of PCL with those of CMAT-FNIII9-10 was constructed and the cellular response of human MSCs was evaluated on this matrix.

Keywords: Recombinant protein; Fibronectin; Real time monitoring; Fusion technology; Double purification; Cell adhesion; PCL; Bio-functionalized

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P34-10

Heterologous secretory expression and characterization of dimerized Bone Morphogenetic Protein 2 in *Bacillus subtilis*

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Human Bone Morphogenetic Protein 2 (hBMP2) has an important clinical applications in the spine fusion and ortho/maxillofacial surgeries. Here we first report the secretory expression of biological active dimerized recombinant hBMP2 from Bacillus substilis system. The mature domain of BMP2 gene was amplified from pTz57R/BMP2 plasmid. By using pHT43 expression vector two constructs; pHT43-BMP2-M and pHT43-BMP2-D, one having single mature BMP2 gene and other with two mature BMP2 genes coupled together with glycine serine rich linker to produce a dimer were designed respectively. The constructs were transformed in DH5α strain and sequence analyzed. For secretory expression analysis and optimization both constructs were transformed in two strains of Bacillus subtilis i.e. SCK6 and WB600. Expression conditions like media and temperature were optimized and maximum 35% and 25% secretory expression of monomer (~13 kDa) and dimer (~25 kDa) respectively was achieved in 2xYT medium at 30 °C in SCK6 strain. The expression and dimeric nature of the hBMP2 was confirmed by western blot and Native PAGE analysis. For the purification of hBMP2, 200 ml culture supernatant was freeze dried to 10 ml, dialyzed against Tris–Cl (pH 8.5) and using Resource Q (6 ml) column, Fast Protein Liquid Chromatography (FPLC) was performed. The hBMP2 monomer and dimer were eluted at 0.9 M and 0.6 M NaCl respectively. The biological activity of dimerized hBMP2 (0, 50, 100, 200 and 400 ng/ml) was analyzed by alkaline phosphatase (ALP) assay on C2C12 cells. The results showed maximum ALP activity at 200 ng/ml in a dose dependent manner.

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P35-1

Structural and functional characterisation of some key fatty acid biosynthesis enzymes and their mode of inhibition by thiourea derivatives

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FAS II pathway comprises many important enzymes assisting in the synthesis of mycolic acids in *Mycobacterium tuberculosis* (Mtb). Most of their structures have been deciphered except one namely, HadBC. It is a dehydratase that brings about the dehydration of β-hydroxyacyl-ACP to trans-2-acyl-ACP thereby aiding in chain elongation. Although HadC bears 53% sequence identity with HadA subunit of HadAB (HadAB is another dehydratase that belongs to the HadABC operon and carries out the same function), it tends to play a unique role in terms of manifesting pathogenicity in the bacterium. Hence, it becomes mandatory for us to unravel what lies beneath the structure that brings about such a vast change in the operational integrity of these proteins. In this regard, we have attempted at solving the HadBC crystal structure at a resolution of 2.9 Å by Molecular Replacement method. Also keeping in mind the recent cases of drug resistance, our study envisages the effect of thiaceatzone (TAC); a prodrug which undergoes biotransforma-tion in the presence of a monooxygenase EthA, thereby acting on HadA. We have devised an *in vitro* methodology to determine the role of EthA as well as MymA (monooxygenase belonging to the virS operon of Mtb) on bioactivation of TAC. Our observation has been substantiated by gel based analysis as well anisotropic studies. Reinforcement of new drug regimen and development of new drug targets has become pivotal. In this scenario, structural analysis and extensive studies encompassing the HadABC complex could serve our purpose.

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P35-2

Crystal structures of maoC dehydratases highlight the substrate regulatory mechanism in *Mycobacterium tuberculosis*

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Fatty acid synthesis (FAS) type II, involved in the mycolic acid synthesis in Mycobacterium tuberculosis, employs a unique maoC like β-hydroxyacyl-ACP dehydratase HadAB or HadBC heterodimer in the third step of the elongation cycle. Alongside, many bypass pathways become operative when Mtb is subjected to stress. HtdX, which is proposed to be involved in bypass fatty acid metabolism pathway, is a maoC like dehydratase which confers antibiotic resistance to the mycobacteria. These dehydratases are one of the least explored enzymes of FAS pathway with respect to its substrate selection. Crystal structure of HadAB complex aided with enzymatic study establishes the roles of HadA as a scaffolding component and HadB as a catalytic component together indispensable for the activity. Additionally, the detailed structural analysis of HadAB in combination with MD simulation endorses the spatial orientation of the central hot-dog helix and the dynamic nature of its associated loop in regulation of substrate specificities. Also, the crystal structure of HtdX in two different forms along with deletion/mutation and MD simulation study, have underlined the regulatory role of α2-β2 loop in substrate binding. We have also investigated the inhibition mechanism of an inexpensive anti-TB pro-drug thiacetazone (TAC) on HadAB complex. This has resulted into the introduction of a promising TAC analogue thereby, unfolding new horizons related to the development of anti-tuberculosis drugs.

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P35-3

Using MicroScale Thermophoresis for the determination of bioremediation proteins in bacteria

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MicroScale Thermophoresis (MST) represents an emerging technology for the determination of biomolecule-ligand binding affinity down to the picomolar range. It is an easy and relatively high-throughput method requiring little sample, and is often cost effective compared to other techniques such as Isothermal Titration Calorimetry and Surface Plasmon Resonance.

In the work presented here we have used MST for studying proteins highlighted during the proteomic analyses of nanoparticle-forming strains of both *Morganella* (Cu) and *Desulfovibrio* (Pt and Pd). We have expressed and purified the proteins then used MST to ascertain if they bind to their respective metals. From this we have enhanced our understanding of the bioremediation pathway in these organisms, which will lead us to the tailoring of each strain for specific bioremediation purposes such as to enhance pathway specificity and to produce more valuable nanoparticles.

Given the ease of which we can carry out MST and the plethora of different bio-molecules it can measure (DNA, RNA, protein, peptides) the scope of what MST can achieve means that it will be a technology commonplace in forthcoming scientific endeavours.

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P35-4

Plasmonic nanoshells based metabolite detection for *in vitro* metabolic diagnostics and therapeutic evaluation

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In vitro metabolite detection relies on designed materials based analytical platforms and is universally employed in biomedical research and clinical practice. However, metabolite analysis in biosamples always needs tedious pre-treatment, due to the sample complexity and low molecular abundance. What is more challenging is to construct diagnostic tools by materials based platforms. Herein, we developed novel platforms using plasmonic nanoshells. We synthesized SiO_2@Ag and SiO_2@Au with tunable shell structures. Optimized nanoshells facilitated metabolome fingerprinting in 0.5 μL of bio-fluids by direct laser desorption/ionization mass spectrometry. We applied these nanoshells for disease diagnosis and therapeutic evaluation. We identified patients with post-operative brain infection through daily monitoring and glucose quantitation in cerebrospinal fluid (CSF). We measured drug dis-

tribution in blood and CSF systems and validated the function of blood–brain/CSF-barriers for pharmacokinetics. Our work sheds light on the design of materials for advanced metabolite analysis and precision diagnostics.

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P36-1

Predicting industrial waste disposal management by using Box–Jenkins ARIMA models: Case study of four waste service providers

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The purpose of this study is to develop forecasting models of four wastes: A waste (Absorbents, filtered waste), B waste (Plastics), C waste (Discarded organic chemicals) and D waste (Sludge from treatment processes). The output of forecast is performed on an Excel application for planning, implementation and assets control as well as physical facilities and financial investments. The waste forecasting models could be used to support the wastes disposal and transportation business of four service providers. The method selected uses Box-Jenkins method with data periods from January 2008 to December 2017 (120 series data). Using Minitab software to analyze the data and fit parameters for models generated, the best forecasting values were by ARIMA (2, 1, 0) or ARI (2,1) for Service Provider A, ARIMA (0, 0, 1) or MA (1) for Service Provider B, ARIMA (3, 2, 2) for Service Provider C and ARIMA (3, 0, 3) or ARMA (3, 3) for Service Provider D. The forecasting of the wastes had RMSE of 467.61, 518.80, 1691.16 and 1102.80, respectively. Suitable forecasting models in Excel application can generate valuable forecasts for service providers to utilize their budget of cash, assets and facilities.

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P36-3

Engineering aspects of biosorption processes applied to the removal of industrial dyes

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Liquid–solid adsorption is at the heart of many separation processes, and the correct design of adsorption plants at pilot or industrial scale requires the characterization of a large vari-ety of elements. It usually starts with the basic physicochemical properties of the adsorbent and then covers the measurement of adsorption capacity (isotherms) and kinetics under different conditions and configurations. Adsorption of Allura Red on granular activated charcoal (GAC) has proven to be a simple, though highly suitable model to validate a systematic approach for the design of adsorptive processes.

The adsorbent capacity was measured and the very steep isotherm was best described by the Freundlich model where $q_{eq} = K_F \times (C_{eq})^n$, with n = 0.19 [–] and K_F = 41.5 [mg^{1-n} L^n g^{-1}]. Kinetics was measured with a dye solution being recirculated through a differential fixed bed of adsorbent. This configura-tion allowed investigating the influence of temperature (5–35 °C), adsorbent particle size (0.10 to >1.1 mm) as well as recircula-tion rate (7–75 mL/min) on the adsorption efficiency. These data were compared with those obtained during batch adsorption trials where the adsorbent was suspended in an agitated solution.

The collected data were analyzed with various models (both empirical and theoretical), which led to the identification of internal pore diffusion as the main adsorption rate limiting step. The values of the mass transfer coefficients that were obtained in the various experiments have been used to predict the outcome of fixed bed trials with a 10 × 150 mm column.

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P36-4

Simultaneous nitrogen and phosphorus removal in an alternating intermittent aeration sequencing moving bed biofilm reactor

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An alternating intermittent aeration sequencing moving bed biofilm reactor (MBBR) was used to treat low strength municipal wastewater. In a pilot-scale plant, the performances of reactor R-I and reactor R-II were investigated by using different sequencing batch operation modes. The results show that the performances

of the two reactors are good. The average effluent concentration of COD, NH3-N, TN, TP and SS in the R-I is 21.23 mg/L, 3.27 mg/L, 5.92 mg/L, 0.55 mg/L and 9 mg/L, respectively, under suitable operation conditions. The R-I is more efficient in simultaneous nitrogen and phosphorus removal and similar in COD removal and solids separation comparing with that of the R-II. Under the condition of low carbon source in sewage, the variable water level operation mode adopted by the R-I is beneficial to improve the utilization rate of carbon source and enhance the performance of nutrient removal. The alternating intermittent aeration MBBR can achieve simultaneous removal of carbon, nitrogen, phosphorus and solids separation in the same physical space of single-stage reactor, reduce aeration consumption and increase the efficiency of simultaneous nitrification and denitrification.

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P36-5

Microalgae-based wastewater treatment and circular economy

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Microalgae are fast growing phototrophic microorganisms with the capacity of utilizing or removing nitrogen and phosphor from aquatic systems very efficiently. Some microalgae are also able to reduce COD of wastewater to a low level under heterotrophic or mixotrophic cultivation conditions. The strength of using microalgae for wastewater treatment mainly relies on their excellent ability to remove N and P from wastewater even in the absence of COD. For the wastewaters containing high content of antibiotics or other micro-pollutants, microalgae-based treatment could be more effective than the conventional bacteria-based activated sludge systems. The microalgal biomass produced during wastewater treatment could be utilized as fertilizers or animal feeds after proper treatments to gain additional benefits via circular economy concepts. The performance and characteristics of using microalgae to treat a variety of wastewaters (e.g., livestock wastewater, fishery wastewater, municipal wastewater, and so on) will be presented. The feasibility and challenges of the microalgae-based wastewater treatment will be discussed. A novel circular economy technology that combines flue gas CO_2 fixation, wastewater treatment with microalgae, and follow-up algal biomass utilization will also be introduced.

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P36-6

Direct current atmospheric pressure glow discharge generated in contact with a flowing liquid cathode as a method for rapid eradication of phytopathogenic bacteria

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Plant pathogenic bacteria are responsible for significant economic losses in crops, vegetables and ornamentals production worldwide. Commonly applied control methods rely on preventive measures, therefore proposal of novel disinfection procedures seems of highest interest. Here, a high-throughput system based on direct current atmospheric pressure glow discharge (dc-APGD) generated in contact with flowing bacterial suspensions acting as a liquid cathode was designed and applied against $OD_{600} \approx 0.1$ (approx. 1×10^7 CFU ml^{-1}) suspensions of *Dickeya solani*, *Xanthomonas campestris*, *Pectobacterium carotovorum*, *Pectobacterium atrosepticum* and *Clavibacter michiganensis*. Eradication efficacy defined as percentage reduction in bacterial population densities enclosed in the range 99.996–100% depending on the species tested and was achieved in less than 1 min. Moreover, physicochemical parameters of the atmospheric pressure plasma including the rotational temperatures of N_2 and OH equaling 2300 ± 100 K and 4200 ± 200 K, electron temperature of 6050 ± 400 K, vibrational temperature of 4000 ± 300 K, and electron number density of 1.1×10^{15} cm^{-1} were determined. The observed antibacterial properties of the discharge were attributed to the formation of numerous reactive species and states in the treated liquid, including NO_x, NH, H_2O_2, O_2, O and OH, in addition to the impact of UVA, UVB, and UVC generated by the dc-APGD source. The proposed applications for this simple, universal, rapid, and cost-effective plasma-reaction system include elimination of plant pathogenic bacteria from industrial and agricultural wastewaters, wastes from 1 to 4 biosafety level laboratories and sterilization of any other liquid disposals putatively contaminated by phytopathogens.

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P36-7

Degradation of bisphenols using immobilized laccase supported onto biopolymer marine sponge scaffolds: Effect of operational parameters on removal efficiency

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Laccases (EC 1.10.3.2) are oxygen oxidoreductases, which catalyse widespread range of chemical reactions, mainly one-electron oxidation of mono-, di- and polyphenols. These enzymes found widespread application in textile, food and medical industry. Besides using of this enzyme in the broadly defined industry, laccase can be use in environmental protection, degrading industrial pollution. Due to its low stability and poor reusability, in recent years, there has been an increasing interest in laccase immobilization, which facilitates possibility to use of laccase at various conditions. A wide range of materials of different origin might be use as supports for enzyme immobilization. Biopolymers are interesting and noteworthy materials, which found application in environmental protection thanks to biocompatibility and biodegradability.

In presented work, spongin-based skeletons of *Hippospongia communis*, were used as a carrier for laccase immobilization. Obtained systems were used for degradation of bisphenol A, bisphenol F and bisphenol S in model solutions. Effective enzyme immobilization and degradation of those hazardous compounds were confirmed by using various methods. Moreover, effects of various initial process parameters, such as temperature, pH and concentration on the removal efficiency of bisphenols were evaluated in this study. In addition, obtained results show that reusability of the laccase was significantly improved after its immobilization.

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P36-8

Use of magnetic halloysite nanotubes-alginate hybrid spheres in the treatment of dyes

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In this study, alginate (ALG) hybrid spheres supported with magnetic halloysite nanotubes (MHNTs) were synthesized. While "co-precipitation" method was used to gain magnetic property to halloysite nanotubes, the synthesis of halloysite-alginate hybrid spheres was done by "dripping" method. In order to determine surface characteristics of these synthesized particles, FT-IR, TGA, SEM, TEM, BET, VSM, particle size and zeta potential measurement analysis were performed. The removal of direct blue 71 (DB71), an anionic dye, by MHNT-ALG hybrid spheres was investigated in batch stirred reactors. The adsorption capacity of DB71 by MHNT-ALG hybrid spheres was compared with that of methylene blue (MB), a cationic dye. With respect to zeta potential measurements, isoelectric point of MHNTs was measured as pH 2.89. At pH values higher than 2.89, MHNT-ALG hybrid spheres carry negative sign. However, a high DB71 adsorption capacity of 365.51 mg/g (5.64 mg/m^2) was obtained at an initial dye concentration of 500 mg/L. This value was comparable to the high adsorption capacity of MB (659.92 mg/g, 8.12 mg/m^2). The adsorption equilibria of dye-stuffs on MHNT-ALG beads were evaluated using adsorption equilibrium models, Langmuir, Freundlich, Redlich-Peterson models. Both the adsorption equilibria of DB71 and MB dyes were represented well by Langmuir type model. The adsorption saturation capacities, q_s, for DB71 and MB dyes, calculated from Langmuir model, were found to be 752.51 and 805.94 mg/g, respectively. The adsorption equilibrium constants, K_L, for DB71 and MB dyes were determined as 0.0025, 0.0316 L/mg, respectively.

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P36-9

Effect of sophorolipid quaternary ammonium salts on activated sludge system

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Sophorolipid quaternary ammonium salts (SQAS) are a new class of biosurfactants obtained as a result of chemical modifications of sophorolipids towards quaternary ammonium salt derivatives. It occurred that some of them possessed stronger antimicrobial properties than the antibiotic gentamicin. In this work four innovative SQAS of antimicrobial properties were tested. The main aim of the work was to evaluate the influence of SQAS on the respiration activity of activated sludge microorganisms and kinetics of biomass growth.

The inhibition of the respiration activity of microorganisms was determined with the use of ToxTrak™ and oxygen uptake rate (OUR) tests. OUR tests were also applied for the determination of the kinetic parameters for the biological treatment of wastewater containing SQAS tested. These were substrate saturation constant (K_S) that regarded the affinity of the substrate to biomass and maximum specific growth rate (μ_{max}) expressing the effect of the SQAS on growth rate of activated sludge biomass.

Both ToxTrak™ and OUR tests showed that SQAS did not inhibit the respiration activity of activated sludge bacteria to the high extent, *i.e.* at the higher level than 50%, when SQAS concentration did not exceed 100 mg l^{-1}. The presence of the studied SQAS did not inhibit the growth of the activated sludge biomass, too. The values of μ_{max} for all SQAS tested were higher than those in the control test (without SQAS). Simultaneously, the elevated values of K_S indicated that the affinity of the substrate for the activated sludge biomass decreased when SQAS was present in the wastewater.

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P36-10

Screening of high flocculant-producting bacteria strain and optimizing of culture conditions for wheat distillery wastewater

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This study aimed to screen flocculant producing bacteria for flocculating suspended matter in wheat distillery wastewater. After preliminary screening and secondary screening, the high efficiency flocculant producing bacteria strain was screened from activated sludge of the wheat distillery wastewater sedimentation tank and the culture conditions of the flocculating bacteria were optimized. A superior strain of Klebsiella M1 was screened by 16Sr DNA biological identification, and the initial flocculation rate was 75.99%. The best fermentation medium components were: Glucose, 15 g/L, peptone, 2 g/L, KH_2PO_4, 1 g/L, and K_2HPO_4, 2.5 g/L. The optimum culture condition was: culture time 48 h, culture temperature 30 °C, pH 4.5, and speed 150 r/min. Under optimum conditions, the flocculation rate was 82.03%. The M1 bacteria strain can be used as the best microbial flocculation of wheat distillery wastewater.

Keywords: Wheat distillery wastewater; Flocculation; *Klebsiella* spp.; Screening; Optimization; Extracting

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P36-11

Use of the *Eichhornia crassipes* as possible biosorbent for the removal of heavy metals from wet limestone flue gas desulfurization plant (WLFGD) wastewater

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The Wet Limestone Flue Gas Desulfurization Plant (WLFGD) process is mainly used to reduce sulphur dioxide content in flue gas emissions so that it falls below the emission limits imposed by respective authorities. WLFGD wastewater produced after the process, has significantly high concentrations of chlorides, magnesium and heavy metals and cannot be re-used in the station. The pollutant content depends on the amount of impurities and heavy metals in the limestone used. To reduce the environmental impact of heavy metals, this research work is focused on *Eichhornia crassipes* as accumulator of divalent heavy metals.

The *Eichhornia crassipes* approach being used in many countries in the World to abstract heavy ions from water. In this study, 145 water samples were collected at the rate two sam-

ples/day at 12 noon and 12 midnight. The samples were stored in labeled beakers of 1 l. For determining the abstraction, 100 ml of the wastewater is mixed with 0.5 g of biosorbent for 45 minutes. The sorption isotherms were measured at 25 °C and analyzed by Langmuir model. The results indicated that the roots of the plant are better accumulators of Pb(II), Cu(II) and Cd(II) than the leaves (up to 96% sorption). On the other hand, the effect of equilibrium pH on sorption showed that the optimal pH value was between 5 and 6. It is also suggested that the *Eichhornia crassipes* could result in a low cost divalent ion metal removal with easy implementation at industrial level.

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P36-12

Synthesis and utilization of self-microstructured TiO_2 nanotube array as novel electrode for wastewater treatment

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The purpose of this research is to investigate the kinetics and reaction mechanism of nitrate reduction on a TiO_2 nanotube electrode in a non-membrane electrolytic system for simultaneous removal of NO_3-N– and NH_4-N. The morphology and crystalline structure of TiO_2 NTs were characterized by scanning electronic microscopy (SEM), energy-dispersive spectroscopy (EDS), Brunauer–Emmett–Teller (BET) analysis and X-ray diffraction (XRD). Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed for studying electrochemical reactivity towards NO_3-N and confirmed that TiO_2 NT electrode has low overpotential and charge transfer resistance. A multiple sets of laboratory-size electrolysis of wastewater containing NO_3-N– and NH_4-N using lab-prepared anodes (IrO_2/Ti, RuO_2/Ti) and cathodes (Ti, Cu, Ni, stainless steel, TiO_2 NT) were performed to study kinetics. The results have shown that the TiO_2 NT anodized under a condition of 60 V, 1.8 A for 15 h, and annealed at 450 °C for 1 h is suitable as cathode for the reduction of NO_3-N and in simultaneous removal of nitrate and ammonia.

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P36-13

Withdrawn