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Scientific Tracks & Abstracts
Day I

Brian G Miller, J Biotechnol Biomater 2017, 7:1(Suppl)
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Allokairic regulation of enzyme function

Brian G Miller
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Human glucokinase (GCK), the body's primary glucose sensor and a major determinant of glucose homeostatic diseases, displays a unique form of allosteric-like behavior that is manifested as a cooperative kinetic response to glucose. The allosteric-like behavior of GCK is particularly intriguing since the enzyme is monomeric and contains only one glucose binding site. Recent work in our laboratory has shown that millisecond timescale order-disorder transitions within the enzyme's small domain govern cooperativity. Here, we present the results of biophysical studies that elucidate the structural and dynamic origins of the time-dependent, allokairic properties of GCK. Using high-resolution nuclear magnetic resonance, we identify two distinct mechanisms by which GCK can be activated, both of which result in hyperinsulinemia. The first activation mechanism alters the equilibrium distribution of GCK conformers in favor of a single-state, whereas the second mechanism alters the intrinsic dynamics of the enzyme without perturbing the relative distribution of states in the structural ensemble. Time-resolved fluorescence measurements map the dynamic conformational landscape of GCK and provide evidence for three distinct conformations of the enzyme in the absence of glucose. Together our findings provide a framework for understanding the origins of time-dependent changes in activity in other regulatory enzymes.

Biography

Brian Miller is an Associate Professor of Biochemistry at the Florida State University, USA. He did his PhD from the University of North Carolina, Chapel Hill in the year 2001. His research interest is protein structure, function and evolution.

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Dejan Bezbradica, J Biotechnol Biomater 2017, 7:1(Suppl)
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Enzymatic synthesis of prebiotic galacto-oligosaccharide: Application of nanobiocatalysts and structural characterization of product

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Statement of Problem: Galacto-oligosaccharides (GOS) are group of β- galactoside compounds with significant market value due to their prebiotic properties utilized in infant nutrition products. Physiological activity is based on their short chain carbohydrate structure which makes them non-digestible by digestive enzymes, but digestible by beneficial probiotic bacteria with consequential property of selective promotion of their growth and improvement of overall health status. State of the art in current industrial GOS production based on transgalactosylation activity of β-galactosidases implies that attempts for further advance could be focused on: Fine-tuning of physiological properties by targeted control of enzymatic process toward obtaining GOS of desired structure and developing novel immobilized β-galactosidase preparations with improved affinity towards GOS synthesis.

Methodology & Theoretical Orientation: For evaluation of the effect of enzyme origin on degree of polymerization and type of β-linkages within obtained GOS compounds, transgalactosylation was performed with different β-galactosidases: from *Aspergillus oryzae* and *Lactobacillus acidophilus*. Elucidation of chemical structures in obtained GOS mixtures was performed using ion-mobility spectrometry–tandem mass spectrometry (IMS-MS/MS) one-step approach. Improvement in the field of β-galactosidase immobilization was attempted by producing novel nanobiocatalyst with functionalized nonporous fumed nano-silica (FNS) particles as immobilization support.

Conclusion & Significance: IMS-MS/MS analysis has shown that structure of obtained GOS is influenced by origin of β-galactosidase, since one from A. oryzae produced GOSs with $\beta(1\rightarrow6)$ and $\beta(1\rightarrow3)$ linkages, while enzyme from L. acidophilus produces GOSs with $\beta(1\rightarrow6)$ and $\beta(1\rightarrow4)$ linkages. Type of glycosidic linkages influences prebiotic properties of GOS, hence determination of linkage type will have great significance in enabling adequate selection of β-galactosidase for targeted prebiotic application. The immobilization on nano-supports indicated that the most adequate support is one functionalized with amino groups, which enabled several times higher transgalactosylation activities than conventionally immobilized β-galactosidase.

Biography

Dejan Bezbradica obtained his PhD degree in Biochemical Engineering and Biotechnology from the Faculty of Technology and Metallurgy in Belgrade in 2007. Since 2013, he is an Associate Professor in the Department of Biochemical Engineering and Biotechnology. During 2009, he was on sabbatical working in the Laboratory of Enzyme Engineering at Institute of Catalysis in Madrid. His scientific work covers following areas: Cell and enzyme immobilization, enzymatic synthesis in microaqueous media, application of membrane reactors in biocatalytic processes; microbial production and purification of industrial enzymes, kinetic modeling of bisubstrate enzymatic reactions, application of enzymes with transglycosylative activity in synthesis of bioactive compounds, chemical modification of enzymes and immobilization supports, and nanobiocatalysis. His recent research activities are focused on the development of food and feed products containing bioactive galactosides with prebiotic activities targeted for specific probiotic species.

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Notes:

The effect of natural deep eutectic solvent on laccase catalyzed polycatechin synthesis

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Catechin is a crucial member of flavonoids that show antioxidant properties both *in vivo* and *in vitro*. However, flavonoid monomers, like catechin, have some disadvantages such as low solubility and pro-oxidant activity. These drawbacks are reported to disappear in the polymerized form. The polymerization of catechin was reported using organic solvents to provide solubility in many studies. We present here the effect of natural deep eutectic solvent (NADES) as green solvents on laccase catalyzed polycatechin synthesis. The reaction media contained catechin (5 mg ml-[1]), acetate buffer (pH=5) and betaine (B)-mannose (M) (5:2, molar amount) at mentioned amounts. The effect of B-M amount (5, 50-90%), laccase concentration (15.6-125 U) and temperature (25-40°C) were investigated on polycatechin synthesis. The antioxidant activities of the polycatechins were tested in terms of superoxide radical scavenging activity and xanthine/xanthine oxidase activity. Size exclusion chromatography and HPLC analysis were used as analytical methods. According to the results, 5% B-M containing reaction media provided high molecular weight polycatechin that was comparable with acetone containing media. Therefore organic solvent content could be discarded from the reaction. However, handling of the reaction media and recovery of the product were challenging steps at increased NADES content. The conversion rate of catechin was found to increase with increasing laccase amount. Additionally, high laccase concentration (125 U) was found to provide high molecular weight and yield. On the other hand, temperature had no significant effect on polycatechin formation at tested range (25-40°C). All polycatechins obtained were found to have increased superoxide radical scavenging activity and xanthine/xanthine oxidase inhibitory activity when compared to monomer catechin. This study showed that polycatechin synthesis pathway could be shifted to a green route using NADES.

Biography

Ayse Ezgi Unlu has expertise in enzymes, enzymatic reactions, fermentation, protein synthesis, proteomics, enzymatic biopolymers and green solvents. The synthesis of Naproxen, a member of NSAIDs, was the subject of her Master's thesis by using commercial lipase subjected to various pre-treatment strategies that enhanced the activity. Investigation of different parameters on the production of lipase by Candida rugosa and also proteomic analysis of the isoenzymes was another subject of her interest. She has done her Post-doctoral research on the synthesis of flavonoids using green solvents.

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Notes:

Mohammed F. El-Shiekha, J Biotechnol Biomater 2017, 7:1(Suppl)
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Biochemical effect of some antioxidants on metabolic changes in experimentally induced tumor in female mice

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Biochemical effect of tannic acid and curcumin on female mice which experimentally induced Ehrlich ascites carcinoma (EAC) was investigated in this study. This study was carried out on 220 (12-14 weeks old, 25-30 g each) female mice. Mice were classified into two main large experiments. Experiment 1: Non-tumor bearing mice (NTB) included 100 animals and divided into four groups, each one comprised 25 mice. Group 1: NTB-control saline treated. Group 2: NTB-treated with curcumin orally (350 mg/kg/day) for 6 weeks. Group 3: NTB-treated with tannic acid orally (160 mg/kg/day) for 6 weeks. Group 4: NTB-treated with curcumin and tannic acid orally at ratio (50%:50%) for 6 weeks. Experiment 2: Tumor bearing (TB) mice. Out of the total 120 animals, were divided into four groups each one comprised of 30 mice. Group 1: TBM-control saline treated. Group 2: TBM-treated with curcumin orally (350 mg/kg/day) for 6 weeks. Group 3: TBM-treated with tannic acid orally (160 mg/kg/day) for 6 weeks. Group 4: TBM-treated with curcumin and tannic acid orally at ratio (50%:50%) for 6 weeks. Blood samples were collected from all animals groups after 2, 4 and 6 weeks from treatment. Serum were separated and processed directly for glucose, insulin, total cholesterol, triacylglycerol, total protein determination. The obtained results revealed that, a highly significant decrease in serum glucose, total cholesterol, total protein concentration, meanwhile, a highly significant increase in serum triacylglycerol concentration was also observed. But a non-significant decrease in serum insulin levels were observed in tumor bearing mice when compared with control. The results of this study indicated that curcumin, tannic acid and their combination treatment have potential benefits in cancer treatment.

Biography

Mohammed F. El-Shiekha has completed his PhD from the Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt. He is a Faculty Member in the Department of Biochemistry, Faculty of Pharmacy, October 6 University, Egypt. He has published 6 papers in reputed journals.

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Metabolic engineering strategies for effective use of glycosyltransferases in oligosaccharide synthesis

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As one of the four building blocks of life, sugar molecules permeate almost all aspects of life. The widespread occurrence of glycosylation and its broad impact in biological processes underscores the importance of studying glycosylation. To study glycans and probe their roles in a biological system significant amount of pure molecules are needed. Besides basic research, there are a wide range of opportunities of utilizing oligosaccharides, polysaccharides and glycoproteins and other glyco-conjugates for diagnosis, vaccine development, as new drug entities and many other medical applications. Unfortunately, these potential applications are all impeded by the lack of large scale synthesis technology for these molecules. Metabolic engineering, since its inception in late 80's, has grown to be a field impactful in the synthesis of a variety of molecules of commercial and societal importance. Opportunities abound at the interface of glycosciences and metabolic engineering. In fact, all sugar moieties in biological components, small or big, free or bound, are important targets for metabolic engineering. Over the past decades, its use in the synthesis of sugar-containing molecules has gained significance. Glycosidic bond formation catalyzed by glycosyltransferase enzyme is in the center of the synthesis of most glycan structures in nature. Oligosaccharides, polysaccharides and glycoproteins share the commonality that requires glycosyltransferases in their synthesis, differing only in the nature of the acceptors. Therefore, from a metabolic engineering point of view, they share much of the synthesis challenges. These include the high energy demand due to the need for sugar nucleotides as precursors, the complexity of metabolic pathways and regulations involved and the adequate supply of acceptors when and where the glycosyltransferases are most active. Represented by 2'-fucosyllactose, the success in bringing highly valuable oligosaccharides to commercial production demonstrates the power of metabolic engineering. On the other hand, given the enormous diversity and significant complexity of saccharide-containing structures, a handful of molecules attaining commercial success can only qualify as a promising beginning. In fact, the surface of the gigantic glyco-sphere has barely scratched. Providing scientists with hundreds and thousands of glycans in quantities sufficient to probe their structure and functional relationships and supplying clinicians with selective compounds (such as Globo H and heparin in Kg quantities) for clinical studies in a cost effective manner are challenges before metabolic engineers and synthetic biologists. The inherent challenges in complex carbohydrates demands innovative metabolic engineering strategies beyond a simple extension of those used in successful examples. In this presentation, metabolic engineering challenges common to glycosyltransferase-catalyzed synthesis of oligosaccharides are analyzed and successful examples from Chen labs are showcased to emphasize the power of metabolic engineering as an enabling technology.

Biography

Rachel Chen has done her PhD from California Institute of Technology in 1994 and subsequently worked as a Research Scientist in Bristol-Myers Squibb. She began her independent academic career in Virginia Commonwealth University and continued at Georgia Institute of Technology. Her research interfaces biology, chemistry and engineering with major focus on applying molecular engineering tools in the synthesis of molecules that are not attainable with conventional means. She has published over 80 peer-reviewed papers and has been serving as an Associate Editor for *Microbial Cell Factories* and on Editorial Boards of *Biotechnology and Bioengineering, AIMS Bioengineering* and *AIMS Microbiology*.

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Inhibition of the RNA-dependent RNA polymerasic activity of *Flavivirus* NS5 by heterocyclic compounds

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Among more than 70 related members of *Flavivirus* genus, Dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), Yellow fever virus (YFV) and Zika virus (ZV) are considered (re)-emerging pathogens that were originally endemic in the tropical regions but recently are spreading also in a wider geographic area. Indeed, there are several environmental, demographic and ecological factors that promote the worldwide diffusion of known and/or novel *flavivirus*es. *Flavivirus*es can produce from mild flu-like symptoms to hemorrhagic fevers, hepatitis and neuropathies, such as encephalopathy, meningitis and microcephaly in human embryos depending on the infective agents. Vaccines are available against YFV, JEV, TBEV and more recently against DENV but the coverage is far from being complete. Moreover, the lack of an effective and specific therapy further worsens the scenario. The RNA-dependent RNA polymerase (RdRp) of the non-structural NS5 protein is one of the most favored targets to find new potential anti-*Flavivirus* drugs. With the aim to find new inhibitors of the RdRp we undertook a research program exploiting, consecutively, two different approaches: i) A virtual screening carried out on the NS5 polymerase domain (DENV RdRp, 2J7U) followed by a biochemical validation on the isolate target, and ii) a direct biochemical screening carried out on DENV NS5 polymerase with the intent to not exclude any potential hit compounds eventually missed during the in silico procedures. Both these approaches were realized using an in-house library of about 200, published and unpublished, compounds previously designed and synthesized as HCV NS5B inhibitors. To validate the potential of the identified hits, an anti-viral activity against a panel of *Flavivirus* was evaluated. The two strategies led us to identify new RdRp inhibitors able to reduce the polymerase activity in the low micromolar range. In particular, the in silico procedure (i) was fruitful for the identification of a pyridobenzothiazole which was extensively characterized with biochemical and structural studies; the second approach (ii) led us to identify functionalized 2,1-benzothiaziens with promising anti-RdRp activity, not emerged as hit compounds during the in silico studies (Figure 1). Also in this case, a representative compound derived from a chemical optimization was better characterized in biochemical and virological assays. The strategy applied in this study led us to identify new promising inhibitors of the NS5 polymerase, worthy of further optimization with the final aim to discover anti-*Flavivirus* agents.

Biography

Giuseppe Manfroni has graduated in Pharmaceutical Chemistry and Technology (2001) and received his PhD in Medicinal Chemistry (2006) from the University of Perugia (Italy). From 2006 to 2008, he worked as a Post-doctoral Researcher at the University of Perugia. From 2008 to date, he is an Assistant Professor in the Department of Pharmaceutical Sciences and is a Lecturer in Pharmaceutical Analysis. He has spent short periods as a Visiting PhD Student at Rega Institute for Medical Research (Leuven, Belgium) and at the Molecular Modeling Laboratory (University of Perugia) under the supervision of Professor Johan Neyts and Professor Gabriele Cruciani, respectively. He is the author of 40 papers and his research is mainly focused on Medicinal Chemistry of antiviral (HIV, HCV, and *Flavivirus*), antitumor and anti-inflammatory (p38 inhibitors) agents. He is an expert in the synthesis of heterocyclic compounds and microwave assisted synthesis.

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Notes:

Zeina Nasr et al., J Biotechnol Biomater 2017, 7:1(Suppl)
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Knockdown of RPS3, a DNA repair endonuclease, impedes colon cancer growth and progression by decreasing lactate dehydrogenase activity

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University of Balamand, Lebanon

Statement of the Problem: In addition to their role in ribosome biogenesis, ribosomal proteins (RPs) play important roles in DNA repair, proliferation, apoptosis and resistance to drugs and chemotherapy. Ribosomal protein S3 (RPS3), a DNA repair endonuclease, is known to be overexpressed in colon adenocarcinoma. In order to ensure their survival, cancer cells rely on aerobic glycolysis catalyzed by the enzyme lactate dehydrogenase (LDH). Our aim is to identify the role of RPS3 in colon cancer growth and metabolism.

Methodology & Theoretical Orientation: Human colon adenocarcinoma Caco-2 and normal colon NCM-640 cells were tested for the expression of RPS3 by Western blot. In order to inhibit RPS3 expression, cells were transfected with siRNA against RPS3 or a non-targeting siRNA (siNT) as a negative control. Upon RPS3 knockdown, cell behaviors were tested including proliferation and survival by trypan blue and WST-1 assays and cell migration and invasion by the Boyden chamber assays. The glycolysis state of colon cancer cells was assessed by measuring LDH activity upon RPS3 knockdown using the LDH assay.

Findings: RPS3 was shown to be expressed in both Caco-2 and NCM-640 cells. RPS3 knockdown in Caco-2 significantly reduced cell proliferation, survival, migration and invasion compared to siNT-transfected cells. In NCM-640, RPS3 knockdown did not significantly affect cell proliferation and survival implying that RPS3 expression is selectively crucial for colon cancer cell growth. Interestingly, LDH activity was suppressed upon RPS3 knockdown, suggesting a decrease in glycolysis which explains in part the decrease in proliferation.

Conclusion & Significance: This is the first report that shows a role of RPS3 in regulating LDH activity therefore affecting the glycolytic state, the survival and proliferation of cancer cells. Our results also demonstrate that RPS3 is a selective molecular marker in colon cancer and a potential attractive target for colon cancer therapy.

Biography

Zeina Nasr is Assistant Professor, at the Department of Biology in the University of Balamand, Lebanon. She did her PhD from McGill University in the Department of Biochemistry. She has her interest in understanding the molecular aspect of tumor initiation and progression. Her research focuses on studying the effect of translation initiation dysregulation on cancer behavior. She has worked with several cell lines and transgenic mouse models and deciphered important pathways that contribute to cancer initiation and progression to metastasis. She has experience in conducting research and teaching at various institutions. Currently, her work focuses on the extra-ribosomal functions of ribosomal proteins and their effects on tumorigenesis.

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Valentina Citro, J Biotechnol Biomater 2017, 7:1(Suppl)
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Pharmacological chaperones for curing enzymopathies: The case of lysosomal alpha-galactosidase

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Pharmacological chaperones are useful for the treatment of enzymopathies arising from mutations that lower the free energy difference between an unfolded and a folded enzyme shifting the equilibrium towards the first form. The unfolded enzyme, although retaining the functional chemical groups is needed for the biological activity, does not maintain them in the appropriate spatial disposition which can be defined as native state. Improperly folded mutant enzymes are usually sensitive to proteolysis and are cleared by the protein quality control systems in the cytosol and endoplasmic reticulum. Activity can be rescued if the equilibrium is pushed back towards the native state. This can be obtained binding a pharmacological chaperone to the folded enzyme. In fact the binding energy of the ligand compensates for the loss in Delta G while unfolding. Lysosomal alpha-galactosidase represents a good model system for the therapy with pharmacological chaperones. Lysosomal alpha-galactosidase catalyzes the removal of α-galactosyl residues from a glycosphingolipid, globotriaosylceramide. Mutations of lysosomal alpha-galactosidase cause Fabry's disease. We used three methods to test the effect of pharmacological chaperones: 1) Thermal shift assay. This test takes advantage of an environmentally sensitive fluorescent dye which binds the enzyme when it reaches the melting temperature; 2) Urea induced unfolding coupled with limited proteolysis and Western blot detection. This test can be carried out on mutants in cell extracts; and 3) Administration of the pharmacological chaperone to cells expressing mutant enzymes. Open reading frames encoding mutated enzymes are introduced into vectors suitable for transient expression. Eukaryotic cells, COS7 or HEK293, are transfected and cultivated in the presence and in the absence of the drug. It can be interpreted that if the chaperones work and the mutants stabilize, a larger amount of protein can be detected by Western blot and consequently a higher enzymatic activity can be measured.

Biography

Valentina Citro is interested in developing Pharmacological Chaperones (PC) to cure rare diseases. She works on the identification of the mutations which can be responsive to chaperones and develop method for assays in vitro in two model systems: The Fabry disease, a lysosomal storage disorder and PMM2-CDG (CDG-Ia) disease, a disorder of glycosylation with no cure at present.

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Internalization of antibody fragments directed against FGFR1

Lukasz Opalinski, Aleksandra Sokolowska-Wedzina, Martyna Szczepara, Malgorzata Zakrzewska and Jacek Otlewski
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Fibroblast growth factors (FGFs) and their plasma membrane-localized receptors (FGFRs) regulate signaling pathways that govern developmental processes and metabolism. Numerous tumors are characterized by the overproduction of FGFR and this is considered a bad prognostic factor for patient survival. Antibody drug conjugates (ADCs) targeting cancer cells with the elevated level of FGFR represent one of the most attractive therapeutic strategies. ADCs are composed of the antibodies raised against tumor-specific biomarkers linked to the highly cytotoxic drugs. After selective binding to the cancer cells ADCs are internalized and delivered to the lysosomes by intracellular vesicular transport system. The lysosomal proteolysis of ADCs results in the release of the cytotoxic drugs, leading to the cell death. A prerequisite for an ADC approach is efficient internalization of the antibody-target complex. Although the biology of FGFRs and their ligands has been broadly studied, the requirements for the effective internalization of antibodies that target FGFR remain elusive. We analyzed the internalization of antibody fragments in various formats that target FGFR1. The antibody fragments in the monovalent scFv format bind to FGFR1, but are not internalized into the model cells that overproduce FGFR1. In contrast, the same scFv proteins in the bivalent scFv-Fc format are efficiently internalized via FGFR1-mediated clathrin and dynamin dependent endocytosis. Interestingly, the receptor kinase function of FGFR1 is dispensable for endocytosis of scFv-Fc-FGFR1 complexes. Binding of the bivalent scFv-Fc induces FGFR1 dimerization without simultaneous receptor activation, suggesting that oligomerization of FGFR1 triggers receptor endocytosis.

Biography

Lukasz Opalinski has completed his MS in Biotechnology from the University of Wroclaw, Poland. In 2012, he obtained his PhD from the University of Groningen, Netherlands. His PhD work was focused on peroxisome proliferation and involvement of peroxisomes in antibiotics production by filamentous fungi. In 2012, he obtained EMBO Long Term Fellowship to study molecular mechanisms of mitochondria biogenesis at the University of Freiburg, Germany. Since 2015, he is working as a Faculty of Biotechnology in the University of Wroclaw, Poland, where he is working on the endocytosis of antibody fragments generated against FGFR1.

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Notes:

Halophilic microorganisms from mural paintings in old Romanian historical monument church and their interactions with nanomaterials

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The mural painting can be bio-deteriorated by micro-organisms in different ways depending on the taxonomic affiliation of micro-organisms, their biology and succession while on a surface. The rate of bio-deterioration is dependent on microclimate conditions, the chemical structure of deposits, the interrelation between bio-deteriogenes and some chemical processes. During colonization of the mural painting surface, some species of micro-organisms synthesized pigments which could be released into the substrate or remain localized within cells but the mural painting surface appears colored. The bio-pigmentation change esthetical appearance of the mural painting or mortar where micro bio-deteriogenes develop. In our case studies (the refectory of Hurezi Monastery and the pre-nave of Humor Monastery), the pink bio-pigmentation is the result of mural painting colonization by halophilic bacteria, namely new strain of *Garicola* genus and some strains of *Halobacillus* spp. They have the ability to grow in media with negligible salt concentration until saturation (extremely halophilic archaea). Taking into account the complexity of salt composition in mural paintings, it appears that these could be a favorable environment from several moderately halophilic micro-organisms. On the other hand, the investigated halophilic micro-organisms showed various growth answers when their culture medium was supplemented with TiO2 nanoparticles. Such kinds of nanomaterials are currently investigated for their potential use in re-saturation procedures of bio-deteriorated historical monuments.

Biography

Madalin Enache is working as a Principal Investigator in the field of Halophilic Microorganisms at the Institute of Biology, Bucharest of the Romanian Academy (IBB). He Graduated from the University of Bucharest in Biochemistry field. Currently, he is also acting as Head of Microbiology Department of the IBB – coordinating research and administrative activities of the Department of Microbiology (IBB); research activities in the fields of Microbiology, Biochemistry, Biology and Ecology. He is involved in coordinating laboratory work, dissemination of the scientific results (scientific papers, participation to conferences and symposia – oral and posters presentations), application for research projects, scientific reports and coordinating projects. He has expertise in various techniques of General Microbiology, Microscopy, Biochemistry and Molecular Biology. His research topics include diversity and phylogeny of halophilic microorganisms; ecology of extremely halophilic archaea, enzymology of halophilic microorganisms and; nanobiotechnology.

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Application of enzymes in the pulp and paper industry

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At Pulp and Paper Institute, enzymatic applications for the main processes in pulp and paper industry i.e. deinking, fibrillation, bleaching and papermaking was investigated in the last years. In our study, we were focusing on improvement of fibrillation and deinking process. During the fibrillation process, objective was to reduce energy consumption while in the deinking process we tried to replace environmentally unfriendly chemicals with enzymes. The deinking is a process for the removal of contaminants from reusable paper fibers. Basically, deinking is carried out in two major phases: The disintegration of printed paper and the separation of ink particles and contaminants from the fibrous suspension by washing or flotation. The enzymatic/neutral deinking is an alternative to counteract the intensive use of chemicals in the conventional process, a process that reduces the environmental impact, efficient and fast, and with which similar results to what has been achieved in deinking using chemical substances are obtained. Paper production is extremely energy-intensive process as 18 to 25% of all the energy needed for the cellulose fibrillation. Cellulose fibrillation is one of the most important processes in the papermaking. This has a major impact on the mechanical properties and, consequently, the mechanical properties of the paper products. The effect of enzymatic treatment (cellulase) on the degree of refining and on the basic, mechanical and optical properties of produced laboratory sheets (thickness, grammage, density, breaking length, tear index, stretch, tensile index, burst index and ISO brightness) was investigated. Enzymatic treatment performed before the refining reduced the refining time for 10%, while treatment after the refining has proven to be ineffective. Enzyme had a positive effect on virtually all measured properties of laboratory sheets made of short eucalyptus`s fibers and slightly inferior effect on sheets produced from long fibers of coniferous. According to results of deinking process efficiency, we can conclude that enzymatic treatment had a positive effect on ISO brightness of all treated samples. Highest ISO brightness was determined after using a mix of enzymes (cellulase, laccase and lipase). Enzymatic treatment had a slightly inferior effect on tensile index of all samples and even the other measured properties were batter with using enzymes.

Biography

Mija Sezun has completed her PhD in Biological and Biotechnological Sciences. Her Doctoral thesis included Environmental Biotechnology area. Currently, she is working at Pulp and Paper Institute and mainly deals with Biotechnology in the paper industry through the use of enzymes in the process of paper production. Currently, her research focuses on the production of enzymes by using fungi and by applying paper mill sludge, as the substrate for the cultivation of fungi. In addition to the fungal enzyme production, she also deals with the use of commercial enzymes to improve the efficiency of processes in the paper industry.

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Microbial bioconversions of biologically active P-C compounds: Scope and limitations of scaling-up

Ewa Żymanczyk-Duda
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Chirality is a crucial feature in the world of living organisms. This is responsible for the specific interactions between biologically active compounds in nature; therefore, it is also fundamental for designing the novel compounds. Among P-C compounds, structures are phosphonate derivatives such as amino phosphonates, keto phosphonates, hydroxy phosphonates. These are analogues of physiological compounds, so they are applied as moderators of activity of particular enzymes involved in natural compounds metabolic pathways. For such purposes, it is necessary to receive them as defined optical isomers. Chemical synthesis of such phosphonates is of low-effectiveness, also for the economic reasons, so biocatalytical approach appeared to be a good alternative. Good results were achieved for the kinetic resolutions of the racemic mixtures of amino- and hydroxy-phosphonates. This was performed via selective oxidation and employing following fungal genera: *Penicillium, Beauveria, Cunninghamella, Verticillium, Cladosporium, Rhodotorula* and *Saccharomyces* (as whole-cells biocatalysts). The same fungal mycelia were able to selectively reduce (thermodynamic process) prochiral keto phosphonates. The above mentioned experiments succeeded based on the laboratory scale and the most effective ones were selected for scaling- up process. This was the hardest part, because it required the modifications of the biotransformation procedures and the biocatalyst form. Experiments were performed with the use of batch and continuous reactors, and the fungal mycelia were immobilized with the use of polyurethane foams.

Biography

Ewa Żymanczyk-Duda has done his MSc in Biotechnology (1990), PhD in Chemistry (1995) from Wroclaw University of Science and Technology, Poland. She has also worked as an Assistant Professor (2008) at the Wroclaw University of Science and Technology, Poland. She was the Vice Dean of Chemistry Department, Coordinator of Teaching Program in the area of Biotechnology. She has published more than 40 papers in reputed journals and has been serving as a reviewer for various journals.

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How to tune recombinant protein production in *E. coli* for enhanced production of biopharmaceuticals?

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Strong induction of recombinant protein production in *E. coli* can lead to agglomeration of inactive product, inclusion bodies (IBs), and also imposes a high metabolic burden which can result in cell death. We developed a feeding strategy using glucose as primary carbon source, lactose as secondary carbon source and inducer to tune recombinant protein expression which leads to higher yields of soluble and active product. We successfully applied this system for the production of several biopharmaceuticals. This new feeding approach allows expression of complex products as soluble and active protein that usually results in insoluble and inactive inclusion bodies. Cell viability and growth can be prolonged by this approach which leads to higher overall yields and thus lower production costs. Thus, our strategy might make *E. coli* a more attractive host for the production of biopharmaceuticals in the future. The audience will get to know a platform technology for the enhanced expression of biopharmaceuticals in *E. coli* to accelerate bioprocess development and yield higher product titers.

Biography

Oliver Spadiut has completed his PhD in Biotechnology from BOKU University, Austria. He has done his Post-doctoral studies from KTH Royal Institute of Technology, Stockholm, Sweden. Since 2010, he has been working as an Assistant Professor in Biochemical Engineering at TU Wien, Vienna, Austria. Currently, he is the Principal Investigator of Integrated Bioprocess Development research group. He has published more than 60 papers in reputed journals and has been serving as a reviewer for many journals.

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Notes:

Cell wall integrity checkpoint protein Rlm1p as novel transcriptional regulator of F-box encoding gene *SAF1* of *S. cerevisiae* during stress

Vijeshwar Verma, Meenu Sharma and **Narendra K Bairwa**
Sri Mata Vaishno Devi University, India

F-box motif containing proteins are shown as the component of protein degradation machinery where they function as substrate recruiting factor. Through substrate recruitment and subsequent degradation of recruited substrate by ubiquitin mediated proteasome machinery they regulate variety of cellular functions such as signal transduction and cell cycle transition. The *S. cerevisiae* cells upon nutrient stress enter into the quiescence stage; at molecular level, this transition is mediated through the recruitment of adenosine deaminase factor Aah1p by the F box motif containing protein Saf1p which constitute the SCF E3 ligase. Here, we have investigated the regulation of the *SAF1* gene by various transcription factors during stress. For this we have analyzed the gene expression profiling database (GEO), transcriptional regulation databases and yeast stress expression database. The gene expression profiling database indicated that *SAF1* gene expression is up regulated during hypoxia and the drug treatment. The subsequent analysis of transcription factors regulating the *SAF1* gene expression revealed RLM1 as novel transcription factor regulating the *SAF1* and its substrate *AAH1* gene expression during stress condition. The yeast stress expression database analysis revealed that in variety of stress conditions (drug, pH, temperature, microbial toxin and inorganic compound) the *RLM1* and *SAF1* were constitutively over expressed at log2 FC>1 and adjusted p-value <0.05 setting in comparison to control cells. The *RLM1* gene has been implicated in the cell wall integrity checkpoint pathway. It has also been reported that RlmA deficient strains lacks cell wall organization and cell growth in A. fumigatus. Based on the analysis we hypothesized that double knockout of *SAF1* and *RLM1* genes cells may be resistant to stress condition which need to be tested experimentally.

Biography

Vijeshwar Verma has completed his PhD in IIIM (former RRL, Jammu) in 1980. Later the institute offered him the position of Scientist and thus he shifted to the Institute in 1982. He was the pioneer member of the group which started a Genetic Engineering Unit in the institute to undertake research in the field of Recombinant DNA in 1986. Presently, he is the Director of School of Biotechnology and Dean of College of Sciences. He is a renowned Researcher in the field of Microbial Biotechnology and has large number of publications and patents to his name. He is a Fellow of Association of Microbiologists of India and Member of Indian National Science Academy. He has spent a significant part of his career at Indian Institute of Integrated Medicine (formerly Regional Research Laboratory) -CSIR, Jammu. This unit later got christened as Division of Biotechnology of which he was the Chairman at the time of taking VRS in 2007. During this period of about 25 years, he had Post-doc experience in the field of Recombinant DNA, Fermentation and General Molecular Biology in various prestigious laboratories/institutes in Germany, England & France, where he learnt a lot about the subject.

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Notes:

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The investigation of effects of commercial protease and *Bacillus subtilis* 168 E6-5 protease on felting and dyeing behaviour of 100% wool fabric

Tuba Sevgi, Elif Demirkan, Dilek Kut and **Meral Dogan**
Uludag University, Turkey

In this study, a novel bacterial strain with high protease activity (210 U/ml) was isolated from soil, and then identified by its morphological character and 16S rRNA sequence, and named *Bacillus subtilis* 168 E6-5. *Bacillus* protease enzyme and commercial protease enzyme were applied to 100% raw wool fabric and bleached wool fabric. After dyeing with acid dyes, changes in the size of the fabric and color yields were measured. Protease was purified by dialysis+lyophilization, and applied on dyed wool fabric and felting shrinkage values were measured. Enzyme treated and dyed wool fabric possess 8%, however non-treated wool fabric has 11% of felting shrinkage value just after dyeing step. After performing five repeated washing, the enzyme treated raw fabric has 12% and the non-treated raw fabric has 15%. After pre-washing, bleaching and dyeing steps, the felting shrinkage value of the enzyme treated wool fabric was 9%, while non-treated one was 11%. After the processes of pre-treatment, bleaching and dying the K/S value indicating the colour yield of the fabric was measued. The K/S value of the wool fabric that was treated with enzyme before the processes of pretreatment, bleaching and dying was 31.68, while the non-enzyme-treated wool fabric has 26.33. Enzyme application increased the colour yield. This study suggests that the *Bacillus* protease enzyme shows better results in behaviours of felting and dying than the commercial protease enzyme and applicable on wool fabrics. Therefore, this protease enzyme has potential in textile industry.

Biography

Tuba Sevgi has completed her MSc from Technical University of Kaiserslautern in Molecular Biotechnology and Systems Biology, Germany. Currently, she is doing her PhD in the Department of Biology, Faculty of Arts and Sciences, Uludag University. She is a Research Assistant in the Department of Biology, Faculty of Arts and Sciences, Uludag University.

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Scientific Tracks & Abstracts
Day 2

Screening of petroleum degrading *Bacillus* spp. strains isolated from different non-contaminated soil samples

Tuba Sevgi, Behice Zeren, Baran Enes Güler and **Elif Demirkan**
Uludag University, Turkey

Oil spill has become a global problem in industrialized and developing countries. Oil spills that occur during discharge from the refineries, accidents of ships/tankers, their grounding, rupture on seabed and on shore pipelines, offshore oil production and exploration platforms do affect these habitats causing irreversible damage to the biodiversity. The toxic effects of crude oil and refined petroleum oils on plants, animals, humans and the environment are devastating. The aim of this study was to evaluate the potential of petroleum hydrocarbon (PHC)-degrading 105 *Bacillus* spp. strains isolated from different non-contaminated soil samples. These *Bacillus* spp. strains were screened for bacterial oil degradation using 3.5% petrol and 7 % diesel as sole carbon sources in Bushnell-Haas agar medium. The plates were incubated at 37°C for 7-17 days. After the incubation, only petroleum degrading bacteria remained on the surface of the plates. Among the 105 *Bacillus* spp. strains, 22 *Bacillus* spp. strains were determined as potential petroleum degrading strains. Most of these strains showed more degradation in diesel medium than petrol medium. Out of the preselected 22 isolates, 18 isolates showed relatively high growth, while 4 others showed moderate to low cell counts after 7 or 17 days of incubation period. This is the first study on *Bacillus* sp. strains isolated from Turkish soils. These isolates seemed to have potential for bioremediation of oil contaminated soil and water.

Biography

Tuba Sevgi has completed her MSc from Technical University of Kaiserslautern in Molecular Biotechnology and Systems Biology, Germany. Currently, she is doing her PhD in the Department of Biology, Faculty of Arts and Sciences, Uludag University. She is a Research Assistant in the Department of Biology, Faculty of Arts and Sciences, Uludag University.

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Notes:

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Identification and characterization of a novel thermo stable and oxidant stable protease from Chumathang hot spring using functional metagenomics

Shafaq Rasool, Vishnu Kumar Gupta, Tishu Devi and **V Verma**
Shri Mata Vaishno Devi University, India

Metagenomics is the genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganism. The common strategies for metagenomics analysis include functional and sequence based approaches. Functional metagenomics is a promising strategy for the exploration of the bio-catalytic potential of micro biomes in order to uncover novel enzymes for industrial processes. However, such methods suffer from low hit rates of positive clones and hence, the discovery of novel enzymatic activities from metagenomes is highly challenging. In the present study, functional metagenomics as a promising approach was applied for exploring the potential of hot springs for various industrial enzymes. Hot spring metagenomics offers the possibility of exploiting the potential of unique niches in order to unravel the functional aspects of the hidden micro biomes. Hot spring metagenomic library of Chumathang-a hot spring of Ladakh region was constructed in *E. coli* using pUC 18 as cloning vector. Functional screening of approximately 10,000 clones was done for protease activity on protease substrate plates. Screening of the metagenomic library led to the identification of one clone with potent protease activity. The clone was designated as pCHpro1. The protease positive clone (pCHpro1) derived from the Chumathang sediment metagenomic library showed 41% identity with subtilase family (sediment metagenome) and 35% structural similarity with crystal structure of Pro-Tk SP from *Thermococcus kodakaraensis*. MEROPS peptidase database analysis showed that it belonged to peptidase S8-S53 superfamily. The enzyme was purified to a final specific activity of 84.51 IUmg-1 proteins with a yield of 15.4%. The purified enzyme had a molecular mass of about ~38 kDa as revealed by SDS-PAGE. The present study indicates that metagenomics without doubt offers the possibility of exploring novel genes/ ORF's which can be characterized and applied in various industrial processes.

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Notes:

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Salt tolerance of potato: Genetically engineered with *Atriplex canescens* BADH gene driven by 3 copies of CAMV35s promoter

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Potato (*Solanum tuberosum L.*) is ranked among the top leading staple food in the world. Salinity adversely affects potato crop yield and quality. Therefore, increased level of salt tolerance is a key factor to ensure high yield. The present study focused on the agrobacterium-mediated transformation of *Atriplex canescens betaine aldehyde dehydrogenase (BADH)* gene, using single, double and triple CAMV35s promoter to improve salt tolerance in potato. Detection of seven potato lines harboring *BADH* gene, followed by identification of T-DNA insertions, determination of transgenes copy no through Southern hybridization and quantification of BADH protein through ELISA were considered in this study. The results clearly depicted that the salt tolerance of potato was found to be promoter-dependent, as the potato transgenic lines with triple promoter showed 4.4 times more glycine betaine production which consequently leads towards high resistance to salt stress as compared to transgenic potato lines with single and double promoters having least production of glycine betaine. Moreover, triple promoter transgenic potato lines have also shown lower levels of H_2O_2, malondialdehyde (MDA), relative electrical conductivity, high proline and chlorophyll content as compared to other two lines having a single and double promoter. *In silico* analysis also confirmed that Atriplex canescens BADH has the tendency to interact with sodium ions and water molecules. Taken together these facts, it can be concluded that over-expression of *BADH* under triple CAMV35s promoter with more glycine betaine, chlorophyll and MDA contents, high relative quantities of other metabolites resulted in an enhanced level of salt tolerance in potato.

Biography

Arfan Ali has completed his PhD from the University of the Punjab, Pakistan and Post-doctoral studies from the Centre of Excellence in Molecular Biology. Currently, he is serving as an Assistant Professor at the University of Lahore, Pakistan. He has published more than 32 papers in reputed journals.

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Special Session
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Zeina Nasr

University of Balamand, Lebanon

The extra-ribosomal role of ribosomal proteins

Protein synthesis is a highly regulated and coordinated process involving the action of ribosomes and a set of translation factors. Ribosome biogenesis occurs in the nucleolus and requires the action of 80 ribosomal proteins (RPs), 4 ribosomal RNAs (rRNAs), other associated proteins and small nucleolar RNAs. The structure of the ribosomal subunits has identified the role of RPs as RNA chaperones for ribosomal assembly and as endo- and exo-nucleases essential for the maturation of rRNAs. Some play a role in the joining of 40S and 60S subunits during translation initiation. Others interact with tRNA or stabilize the ribosome by encasing the exit groove. Studies have also shown that RPs may have extra-ribosomal functions, ranging from DNA repair to replication, proliferation, apoptosis and chemoresistance. Mutations in RPs in animal models and humans induced a wide variety of phenotypes suggesting a role of RPs beyond the ribosome structure. Thus far, 11 RP mutant mice have been reported exhibiting diverse phenotypes including decrease body size, defective organs and embryonic lethality. Defects in ribosome biogenesis have been linked to many diseases collectively named ribosomopathies. These include myelodysplastic syndromes, due to *RPS14* haploinsufficiency and Diamond-Blackfan anemia, caused by mutations in *RPS19* gene. These abnormalities have shown an increase susceptibility to hematological malignancies. Indeed, RPs has been linked to tumorigenesis in several reports, suggesting a role in promoting transformation. Several RPs are overexpressed upon activation of the oncogene *Myc*. Some are found overexpressed in various human tumors, including prostate and colon cancer, metastatic nasopharyngeal carcinomas, metastatic melanomas and metastatic human breast cancer cells. Some RPs has also been proposed as biomarkers for various cancers, such as colorectal, gastric, prostate cancers and lymph node metastasis. These evidences suggest that RPs could be used as potential targets in cancer therapeutics.

Biography

Zeina Nasr is Assistant Professor, at the Department of Biology in the University of Balamand, Lebanon. She did her PhD from McGill University in the Department of Biochemistry. She has her interest in understanding the molecular aspect of tumor initiation and progression. Her research focuses on studying the effect of translation initiation dysregulation on cancer behavior. She has worked with several cell lines and transgenic mouse models and deciphered important pathways that contribute to cancer initiation and progression to metastasis. She has experience in conducting research and teaching at various institutions. Currently, her work focuses on the extra-ribosomal functions of ribosomal proteins and their effects on tumorigenesis.

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Notes:

The catalytic and structural roles of the Human Hexokinase 2 in cancer

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Glucose metabolism is 200 times higher in cancer affected tissues in comparison to normal tissue as a strategy to support tumor growth and progression, historically known as the 'Warburg effect'. Hexokinase is the first enzyme of the glycolytic pathway that catalyzes the phosphorylation of glucose for its activation to glucose-6-phosphate and uses ATP as high-energy source of phosphates. Four isozymes are present in human body with hexokinase 2 (HK2) as most active and specifically expressed in variety of different cancers. However, HK2 binding to the outer mitochondrial membrane not only gives it prime access to ATP generated by the mitochondrial but inhibit apoptosis. Here, we aim to biochemically and structurally characterize interactions of HK2 with the mitochondria and the N-terminal role in catalysis and stability of the full-length enzyme. Here, we solved the crystal structure of human HK2 in complex with glucose and glucose-6-phosphate (PDB code: 2NZT), where it is a homodimer with catalytically active N- and C-terminal domains linked by a seven-turn α-helix. Different from the inactive N-terminal domains of isozymes 1 and 3, the N-domain of HK2 is not only capable of catalyzing reaction but it is also responsible for thermodynamic stabilization of the full-length enzyme. Deletion of first α-helix of the N-domain that binds to the mitochondria altered the stability and catalytic activity of the full-length HK2. In addition, we found the linker helix between the N- and C-terminal domains to play an important role in controlling the catalytic activity of the N-terminal domain. HK2 is a major step in the regulation of glucose metabolism in cancer making it an ideal target for the development of new anticancer therapeutics. Characterizing the structural and molecular mechanisms of human HK2 and its role in cancer metabolism will accelerate the design and development of new cancer therapeutics that are safe and cancer specific.

Biography

Wael M Rabeh has received his PhD in Biochemistry from the Lab of Professor Paul F Cook, where he characterized the last enzymatic reaction of the cysteine biosynthetic pathway in *Salmonella typhimurium*. In 2005, he joined the Structural Genomic Consortium (SGC) at the University of Toronto as a Post-doctoral Fellow, where he characterized the 3D structure of human proteins with medical relevance using X-ray crystallography. In 2007, he joined the Lab of Dr. Gergely Lukacs at McGill University for the characterization of a membrane channel that is the main cause of cystic fibrosis. His research focuses on the characterization of protein structures and mechanism to understand their biological functions.

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Notes:

Upstream and downstream processing of fungal laccase

Anna Antecka[1], **Michal Blatkiewicz**[1], **Pawel Gluszcz**[1], **Stanislaw Ledakowicz**[1] and **Andrzej Gorak**[1, 2]
[1]Lodz University of Technology, Poland
[2]Dortmund University of Technology, Germany

Statement of the Problem: Laccase (EC 1.10.3.2, polyphenol oxidases) belongs to the group of oxidoreductases which is characterized by its specific catalytic properties and the ability to oxidize various organic compounds. Therefore the enzyme is very attractive for a wide range of industrial and environmental purposes. However, due to relatively low effectiveness and the possibility of gradual degradation of bioproducts in the reactor or during the separation and purification stages, there is a need for new approaches and research in this field. Therefore, the purpose of this research was to study and integrate the stages of up- and downstream processing (biosynthesis and purification) of laccase from *Cerrena unicolor* in order to obtain a highly active enzymatic product.

Methodology: The biosynthesis was performed in a 14 L bioreactor equipped with a set of sensors for process control. Modifications to the medium (addition of microparticles), MPEC, as well as various types of cultivation/growth strategies were examined. The supernatant was concentrated and purified by an aqueous two-phase system (ATPS) consisting of polyethylene glycol and phosphate buffer solutions and through foam fractionation (FF) at different pH values and with the addition of different detergents. Ultrafiltration and chromatography methods were also investigated. Molecular mass and isoelectric point was determined with the use of electrophoresis.

Findings: Laccase activity increased 3.5-fold after addition of microparticles to the culture media. The fed-batch mode resulted in high laccase activity (up to 4 U/mL) which remains stable during cultivation. The optimal conditions for laccase purification by FF and ATPS were determined with activity partitioning coefficients between foamate and retentate of almost 200 and 2000, respectively, and with yields reaching 50% and 90%, respectively.

Conclusions: Application of MPEC and fed-batch mode proved successful in increasing enzyme production. Hence, both ATPS and FF can be used for laccase purification.

Biography

Anna Antecka has received her PhD in Environmental Engineering in 2008 from the Lodz University of Technology in Currently, she is an Assistant Professor in the Department of Bioprocess Engineering at Lodz University of Technology. From 2004-2005, she has worked at the International Institute Zittau, Germany, in the Department of Environmental Biotechnology. In 2002, she studied at the University of Dortmund, Germany. Her main research interests are in microbial ecology and biotechnology of fungal enzymes especially laccase, including its production, purification and characterization, as well as enzyme applications for industrial and environmental purposes. Currently, she is working in the area of integrated continuous up- and downstream processes for the biosynthesis and purification of fungal laccases.

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Continuous methods of fungal laccase concentration

Michal Blatkiewicz[1], **Anna Antecka**[1], **Stanislaw Ledakowicz**[1] and **Andrzej Gorak**[1, 2]
[1]Lodz University of Technology, Poland
[2]Dortmund University of Technology, Germany

Statement of the Problem: Downstream processing of biological molecules is a very time- and energy-consuming task. One of the major trends in contemporary biotechnology revolves around cost-effective and environment-friendly methods of concentration and purification of bioproducts. Various novel downstream processing tactics are currently being investigated as alternatives to established methods such as ultrafiltration and chromatography. The purpose of this research was to examine the feasibility of polyethylene glycol-phosphate aqueous two phase systems (ATPS) and cetrimonium bromide-induced foam fractionation (FF) as methods for *Cerrena unicolor* and *Pleurotus sapidus* laccase separation from culture supernatants. Both processes were investigated in batch and continuous forms.

Methodology: The biosynthesis was performed in a 14-L bioreactor equipped with a set of sensors for process control. The filtered supernatants were concentrated with the use of aqueous two-phase systems or foam fractionation. Batch ATPS experiments were conducted in specially designed extraction flasks, and for continuous ATPS experiments, a mixer-settler unit (MSU) was used. FF experiments were conducted in a special glass column equipped with air disperser and peristaltic pumps for liquid intake and outtake. Laccase activity was determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay.

Findings: *C. unicolor* laccase showed greater affinity towards salt-rich phase with over 90% yields and partitioning coefficients up to 2200. *P. sapidus* laccase showed strong affinity towards polymer rich-phase also with over 90% yields and full partitioning. MSU experiments showed consistency with batch experiments within non-extreme phase ratio range. Foam fractionation effectiveness depended strongly on pH and surfactant concentration, leading over 100 partitioning coefficient towards the foamate. Low gas and liquid flow rates led to more effective concentration.

Conclusions: Aqueous two-phase extraction and foam fractionation are both effective alternatives to established downstream processing methods for laccase concentration.

Biography

Michal Blatkiewicz has done his Master of Engineering Technology in the field of Chemical and Process Engineering from Cracow University. Currently, he is a PhD student at Lodz University of Technology, Faculty of Process and Environmental Engineering, where he is also employed as a Scientific Project Contractor. During his PhD studies, he has done four internships at Dortmund University of Technology. His scientific scope includes primarily fungal cultures and enzymes, and also downstream processing of biological molecules. Currently, he is a part of a research team working on a project concerning continuous processes of biosynthesis, concentration, and purification of fungal laccases, in which he focuses mostly on novel downstream processing methods, such as aqueous two-phase extraction and foam fractionation.

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Thermophilic enzymes as industrial biocatalysts

Jennifer A Littlechild
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There is an increasing demand for new enzymes with enhanced performance and/or novel functionalities that provide savings in time, money and energy for industrial processes in the areas of high value chemical production and other "white" biotechnology applications. There is limited understanding of the metabolic capacity of life and only a small proportion of nature's catalysts have been utilised for industrial biotechnology. There are new metabolic pathways and enzyme activities to be discovered and many of which could be identified within the large proportion of micro-organisms that cannot be cultured and within their associated viruses. The number of enzymes explored to date remains within the range of 1-2% of known microbial diversity. Enzymes used for commercial biotransformation reactions are required to be stable under the industrial conditions employed. The use of naturally thermostable enzymes isolated from hot environments can be a source of enzymes that are more stable to high temperatures, extremes of pH and exposure to organic solvents. By using both genomic and metagenomic approaches within the projects, HotZyme and THERMOGENE, we have identified hydrolase and transferase enzymes of industrial interest isolated from high temperature environments around the world. A selection of these novel enzymes including esterases, cellulases, epoxide hydrolases, transketolases and transaminases have been characterized both biochemically and structurally. In case of the epoxide hydrolases, two new enzymes with interesting substrate specificity and stereo-selectivity have been discovered from thermophilic metagenomes. Applications of these new epoxide hydrolases have been demonstrated at industrial scale for the production of new chiral chemical building blocks. A new thermophilic cellulase enzyme with activity at pH 5.0 and active under high salt conditions has been isolated which has potential applications for breakdown of biomass.

Biography

Jennifer A Littlechild is a Professor of Biological Chemistry and has established the Henry Wellcome Centre for Biocatalysis at Exeter University in 2003. Her research studies involve the structural and mechanistic characterisation of a range of enzymes from thermophilic bacteria and archaea that have industrial applications. She has a particular interest in thermophilic carbonic anhydrase enzymes and has carried out a project with Statoil from 2011-2013. She has published over 200 publications in high impact journals and has presented her research work internationally. She is the UK Representative and Vice-Chair of the European Section of Applied Biocatalysis and member of EU Advisory Committee for Industrial Biotechnology.

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Young Research Forum
Day 2

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The role of zeolite in reducing oxidative damage in tomato plants exposed to drought

Dino Hasanagic[1], **Danijela Kojic**[2] and **Biljana Kukavica**[1]
[1]University of Banja Luka, Bosnia and Herzegovina
[2]University of Novi Sad, Serbia

Statement of the Problem: The drought is a worldwide problem and insufficient supply of plants with water is one of the most important causes of low agricultural yields and thus representing one of the most common problems faced by the producers. There has been an increased interest in science in recent years in the use of natural aluminosilicates in agriculture where the most famous is zeolite, a mineral whose absorption properties and balanced release of water and nutritive substances ever more successfully solve the issue of water supplying and mineral nutrition and have beneficial impact on overall plant growth. The aim of this study was to investigate the role of zeolite in prevention of oxidative stress in tomato plants exposed to drought. Changes in the activity of peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), superoxide dismutase (SOD, EC 1.15.1.1) as well as reduced and total ascorbate content in plant leaves exposed to drought for 28 days were investigated. Activities of antioxidant enzymes in the leaves of plants exposed to drought were at the same level with and without the addition of zeolite. The obtained results indicate that zeolite did not prevent oxidative damages caused by drought. Native electrophoresis resolved the presence of two peroxidase isoforms specific for drought and their activities were higher in tomato leaves whit zeolite. The drought induced an increase in activities of superoxide dismutase, ascorbate peroxidase and ascorbate concentration and this antioxidative strategy was more expressed in zeolite treated plants. Unexpected results related to the role of zeolite open the possibility to different perspectives in discussion on the zeolite role in drought prevention.

Biography

Dino Hasanagic received his degree of M.Sc. from the Faculty of Science in Sarajevo, and he is currently attending plant biochemistry programme at Faculty of Science in Novi Sad, Serbia. His field of interest is antioxidative metabolism of plant cell - effects of abiotic stress (drought, metals, senescence, ROS signaling, redox state). Dr Biljana Kukavica is expert in plant enzyme antioxidative metabolism. Her interest is focused on enzyme and non-enzyme antioxidative defense in plant cell under oxidative stress. Dr Danijela Kojic has a big experience in the field of biochemistry and molecular biology and her researches are addressing the antioxidative enzyme role in adaptation to stress conditions. The expertise and passion of the authors is recognized among their peers, and they constantly expanding their knowledge in researches regarding enzymology.

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Electroconducting π-conjugated N-nonylaryl oligomers as a matrix in the construction of laccase biosensors

Agnieszka Jedrychowska, Joanna Cabaj and **Jadwiga Soloducho**
Wroclaw University of Technology, Poland

Biosensors found the extensive applications in monitoring of atmospheric pollution and contamination of soil and water, medical diagnostics, analysis of food products. Although, for many years extensive research have been carrying out in the field of sensors, there is still a great need to develop low-cost and efficient diagnostic equipment, enabling fast and accurate detection of the analyte. The crucial issues in the design of enzymatic electrodes are: To ensure the efficient charge transfer between the active site of the biocatalyst and the electrode surface and to provide strong and long-lasting binding between the matrix and the enzyme, without a negative impact on the catalytic activity of the protein. Since the use of electroconducting materials in construction of biosensors improves the devices performance, the purpose of the researches was the chemical modification of substrates with new π-conjugated oligomers and the efficient immobilization of laccase to the prepared semi-conducting matrices. The thin layers of heterocyclic N-nonylaryl polymers based on 2,5-pyridine were prepared on glassy substrates by spin-coating and visualised by atomic force microscopy. The measurements of the catalytic activity of the immobilized enzyme were carried out using a colorimetric method. The presented studies show prospective application of developed systems with immobilized laccase for biosensing purposes, i.e. for the detection of phenolic compounds in food products or for the environment monitoring.

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Biography

Agnieszka Jedrychowska is pursuing his PhD in Faculty of Chemistry at Wrocław University of Technology. Her research interests include "Properties of well-ordered, thin films built of a new class of heterocyclic conducting compounds for the construction of simple sensors". Her most important scientific field concerns the efficient immobilization of oxidoreductases onto chemically modificated substrates and the measurement of the catalytic activity and determination of the stability of the immobilized enzymes in such systems. She is a co-author of 11 publications, six patents and five patent declarations.

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Notes:

Biological oxidation in organic synthesis

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Oxidation reactions are a central and general field of organic and industrial chemistry, being defined as: "a process which involves a gain in oxygen and/or loss of hydrogen". In recent years, a wide range of oxidative procedure has been developed in the attempt of synthetic chemist to satisfy the need of reaction efficiency and with regard of environmental impact. Although a great effort has been made, the development of new synthetic green processes is desirable. In pursuing our interest in green chemistry combined with our expertise in the selenium chemistry we have developed new eco-friendly oxidative process taking inspiration by biological mechanism of glutathione peroxidase (GPx). The catalytic cycle of GPx is an oxidation reaction in which the selenium atom of a selenocysteine is the catalyst; the hydrogen peroxide is the stoichiometric oxidant, and glutathione the substrate that is subjected to oxidation. In the native enzyme the catalytic triad prevents over oxidation of selenium that is able to oxidize only the thiols. In the absence of thiols and in the absence of stabilizing interaction small selenium containing compound are oxidized to selenonic or perseleninic acid and in this form they can transfer oxygen to carbon-carbon double bond and carbon oxygen double bond of aldehydes. Interestingly, these reactions can be carried out in "on-water" conditions having as unique side product a molecule of water. Herein we present some example of "bio-logical" oxidation applied to unsaturated organic substrates for the synthesis of diol, oxidation of aldehydes into carboxylic acids and oxidative cyclofunctionalization. We used catalytic amount of selenium based catalyst, as selenocysteine and diphenyl diselenide, and hydrogen peroxide as stoichiometric co-oxidant as greener oxidant. Furthermore, we use mild condition, and we could recycle the catalyst at least five times without an evident loss in terms of yields.

Biography

Francesca Mangiavacchi has received her Master's degree in 2016 under the supervision of Professor Antimo Gioiello and Claudio Santi in the Department of Pharmaceutical Sciences of University of Perugia. Currently, she is a Research Fellow in the group of Catalysis and Organic Green Chemistry of Professor Claudio Santi working on the development of new catalytic methods for oxidation of natural derivatives under mild conditions. In 2015, she received the award for the Best Oral Communication at the 4th Workshop on Selenium Sulphur and Redox Catalysis and in 2016 a Travel Fellowship from the University of Melbourne to attend at the International Conference on Selenium and Tellurium in Gifu (Japan) under the project Selenium (Redox) Therapeutics.

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Investigating miRNA-661 and ATG4b mRNA expression as potential biomarkers for hepatocellular carcinoma

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Aim: In this study, we aimed to examine the statistical association of serum expression of miR-661 and ATG-4b mRNA with HCC based on *in silico* data analysis followed by clinical validation.

Patients & Methods: Bioinformatics prediction was first applied to retrieve the potential miR serving as an epigenetic regulator of ATG-4b mRNA. Real-time quantitative polymerase chain reaction(RT-qPCR) were used to examine the expression of miR-661 and candidate target gene ATG-4b mRNA in 105 hepatocellular carcinoma (HCC) patients, 50 chronic hepatitis C infected (CHC) patients and 45 healthy controls. The prognostic efficacy of the chosen genes was also explored.

Results: The expression of miR-661 and ATG-4B mRNA was positive in 97.14% and 77.14%, respectively. HCC patients with strong discriminating power between HCC and control showed AUC=0.9 and 0.8, respectively. The median follow up period was 28 months. The survival analysis showed that ATG-4b mRNA was not dependent on the prognostic factors. We also found that miR-661 was positively correlated with ATG-4b mRNA in patients 'sera samples.

Conclusion: This is the first report about the considerable clinical use of miR-661 and ATG-4b mRNA in early detection and follow-up of HCC patients.

Biography

Osama Saber is a second year Medical Cadet in Armed Forces College of Medicine (AFCM). He is a member of International Genetic Engineering Machine Foundation. He has published many papers in various reputed journals.

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