

Sector of

Applied Biocatalysis



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Sector of Applied Biocatalysis was established in 2007 as a group of Industrial Biotechnology in conjunction with the start of the National Programme on the Development of Industrial Biotechnology in Lithuania 2007-2010. In 2010 the group was transformed into the Sector of Applied Biocatalysis and is headed by Inga Matijošytė (PhD in biochemistry and biocatalysis from Delft University of Technology, The Netherlands, 2008). The limited number of suitable and well characterized biocatalysts delays the progress in the application of enzymes in the synthesis of compounds for materials, pharmaceuticals and chemicals. Sector's research is directed towards the search for enzymes with new functionalities and their development towards applied biocatalysis. The research focuses on developing of biocatalytic systems employing oxidative, lyolytic, hydrolytic, proteolytic enzymes. Recently, the sector is orienting the research towards discovery of new novel biocatalytic routes for high-added value products from bio-based raw materials – biopolymers. We strive to meet scientific challenges in combination with application-oriented research.

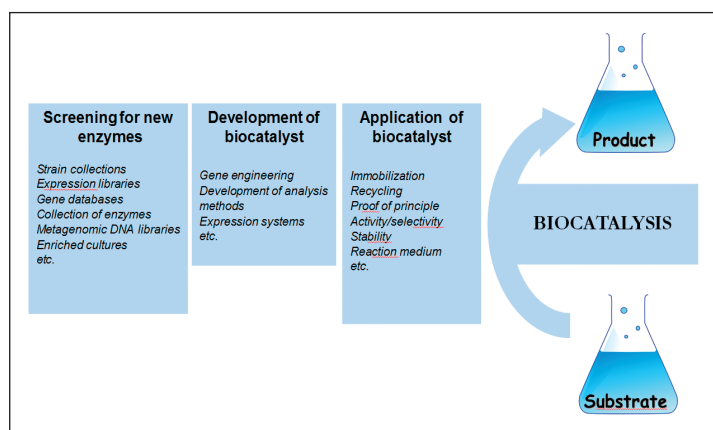


Figure 1. Research areas of Sector of Applied Biocatalysis. Sector of Applied Biocatalysis seeks to identify biocatalysts with novel activities by screening for enzymes, development of biocatalyst and application of biocatalyst

Screening for new enzymes

• Metagenomic DNA libraries

The demand for enzymes with new or specific characteristics and functionalities is constantly increasing. A vast majority of microbial species producing enzymes of interest are living in some unique environments and it is difficult or sometimes impossible to cultivate them at the laboratory conditions. Well known that less than 1 % of the microorganisms found in the nature could be cultivated under the laboratory conditions, that is why only a

miserable part of their genetic potential can be covered by standard microbiological methods. Metagenomics approach is one of the modern methods for searching and investigation of new enzymes, thus, reducing the cultivation problem. This method is based on the direct extraction and analysis of total DNA from the environmental samples. We are able to produce high quality metagenomic DNA libraries from DNA originated from sludge, soil and polluted water samples. Functional analysis of some constructed libraries indicated presence of lipase, hydrolase, esterase, peroxidase, epoxidase and laccase activities. Sequencing of some inserts revealed the presence of enoyl ACP reductase, 1-deoxy-D-xylulose-5-phosphate synthase (DXP synthase), chorizmate mutase/prephenate dehydrogenase, diaminopimelate decarboxylase, acetyltransferase, NADH dehydrogenase (ubiquinone), histidinol phosphate amino transferase.

• Microorganisms from environmental samples

Another way for the discovery of microorganisms with distinguished features is accomplished by the enrichment cultivation of the environmental samples on the delicately selected substrates. Using this approach we have already isolated *Bacillus pumilus*, *Pseudomonas pertucinogena*, four strains of *Pseudomonas putida*, four strains of *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Alcaligenes sp.*, etc. Valuable enzymes such as secondary alcohol oxidase, laccase, cytochrome C oxidase, lipase were identified in the isolated microorganisms.

Development of biocatalysts

• Gene engineering

The project BIOLUBRICANT was performed in collaboration with a company JSC Biocentras with the main goal to develop a biocatalyst – lipase for the production of oil based lubricant, due to the price of commercial lipase which effects the whole process significantly. The developed and constituted purification scheme for lipolytic enzymes using single-step purification enables to reduce costs and consumption of reagents and energy, which are needed for purification procedures in a large scale. A new bacterial lipase from *Serratia sp.* was cloned. Bacterial gene was synthesized de novo by GenScript, using OptimumGene™ codon optimization technology. The new gene was translocated into the original heterologous protein expression system in the yeast *Kluyveromyces lactis*, based on a strong promoter and signal

peptide usage. In both cases, the attempts gave desirable results – only extracellular lipase activity was determined. Following studies for estimation of optimal cultivation conditions showed that *K. lactis* can be successfully grown in the medium containing industrial waste the whey – a cheap raw material. Furthermore, both variants of lipases (mG1 and sG1) expressed in *K. lactis* showed promising characteristics for application in biocatalytic systems. Also, our study showed that de novo synthesis technology used for production of targeted biocatalyst directly from genome sequence data has undoubtedly the future: it can improve the expression level in the system of choice for production of sufficient amount of biocatalyst; also, it may save time required for screening and isolation of enzymes.

● *Expression systems*

Several systems for the expression of the genes of the metagenomic origin is employed in our laboratory so far - in *E. coli* and in unique heterologous protein expression system in yeast. The latter let us express heterologous genes in several yeast species, including *Kluyveromyces lactis*, *Kluyveromyces marxianus* and *S. cerevisiae*.

● *Development of analysis methods*

Enzyme collections are typically of environmental origin, such as microbial strain collections and/or metagenomic libraries. Screening systems are classified into three different classes: i) growing cells (colour test, genetic selection, etc.); ii) microtiter plate/microarray (enzyme coupled assays, labeled substrates, product sensors); iii) instruments (GC, HPLC, TLC, isotope labels, etc.). We are keen on development of screening systems for growing cells aiming to search for targeted enzymes. Enzyme assays based on coloured substrates or products can be applied to identify colonies producing active enzymes which can be then be picked for future studies. We have developed such assays for epoxidation, hydrolysis, lipolytic, oxidation (laccase) enzyme activities. Also, we are developing direct and indirect assays for soluble and immobilized enzyme activities or identification of different functional groups of various compounds using Oxygraph instrument. It was designed and optimized assay for phenol functional group determination in fat, oil and aqueous originated samples. Also, it was developed an assay for free fatty acid determination in fats and oils. The assay for determination of the amount of reduced sugars was adopted for different stages in biodiesel production process.

Application of biocatalyst

● *Synthesis of biopolyols*

Traditional bulk chemical manufacturing abounds with classical “stoichiometric” technologies. Conversely, the introduction of biocatalytic reactions is well suited to cost reduction and higher quality products, as well as to more sustainable processes. As safety, health and environmental issues are key drivers for process improvements in the chemical industry, the development of biocatalytic reactions or pathways replacing hazardous reagents is a major focus. The aim of this project is to improve the economic aspects of a green chemistry-based polyurethane production, whereas polyols are important starting materials for the manufacture of polyurethanes. We have developed a chemo-enzymatic synthesis reaction involving epoxidation and hydrolysis. For the first step commercial immobilized lipase (Novozym 435) is applied and for the second step commercial ion exchange resin based catalyst Amberlyst 15 is used. Application of other enzymes with epoxidizing and hydrolyzing activities can achieved more economical and environmental benefits for biopolyol production. Recently, we have isolated several microorganisms from environmental samples which are able to synthesize polyols from rapeseed oil. The research is directed towards characterization of particular enzymes catalyzing this reaction and scale-up optimization of the process.

● *Immobilization*

Immobilization allows for an easy recovery and subsequent reuse of enzymes, thus further reducing the costs of enzymatically catalyzed processes. In general, immobilization is classified into the main three methods: i) binding to a support (carrier), ii) entrapment (encapsulation) and iii) cross-linking. PhD student Milda Šulcienė during implementation of the collaborative SCIEX project at University of Applied Sciences (Switzerland) performed immobilization of enzymes with epoxidizing and hydrolyzing activities on solid surface of amino-modified silica nanoparticles and the subsequent covalent cross-linking using two linkers: glutaraldehyde and triacryloylhexahydrotriazine. Seven objects were chosen for immobilization: four bacterial strains (*B. pumilus*, *P. pertucinogena*, E10 and E12 (from metagenomic library) and three commercial yeast strains (*Y. lipolytica* DSMZ 1345, *Y. lipolytica* DSMZ 3286, *C. cylindracea* DSMZ 2031). Optimal conditions for

immobilization of samples from bacteria and yeasts origin were determined. Influence of carrier, pH, sorption time, concentration of linker, cross-linking time for immobilization were estimated. Further, the stability of immobilized derivatives under optimal conditions was investigated.

We are also developing a carrier-free immobilization methods

(CLE, CLEA, „layered“ CLEA). The studies on immobilization of wild type lipase from *Serratia* sp. and its prototypes by CLEA method revealed that it can be successfully applied for the biocatalytic synthesis of 2-ethyl-1-hexyl oleate (biolubricant). Immobilization of alcohol oxidase from *Pichia pastoris* by CLEA method was initiated and is under development.

Collaboration

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Publications 2013-2014

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Book Chapter

Buchovec I., Pamedytyte V., **Gruskiene R.**, Luksiene Z. Novel approach to the microbial decontamination of wheat sprouts: photoactivated chlorophyllin-chitosan complex // Industrial, medical and environmental applications of microorganisms. Current status and trends. ISBN: 978-90-8686-243-6. Editor: Antonio Mendez-Vilas. Wageningen Academic Publishers **2014**, 352-356.