P-18 Jarosław Popłoński

Evaluating the Efficacy of a Sucrose Synthase-Based In Vitro Cascade for the Biosynthesis of Flavonoid Galactosides

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Sucrose synthase (SuSy) is a glycosyltransferase found mainly in plants but also in bacteria. The enzyme catalyses the reversible transfer of a glucosyl moiety between fructose and a nucleoside diphosphate. The main biocatalytic application of this enzyme lies in easy and fast production of NDP-glucose from sucrose, what is commonly used in cascade reactions with other glucosyltransferases [1]. There are many described applications of this cascade [2], however, the introduction of enzymes capable of modification of NDP-glucose within the cascade enables broader utility of this enzyme.

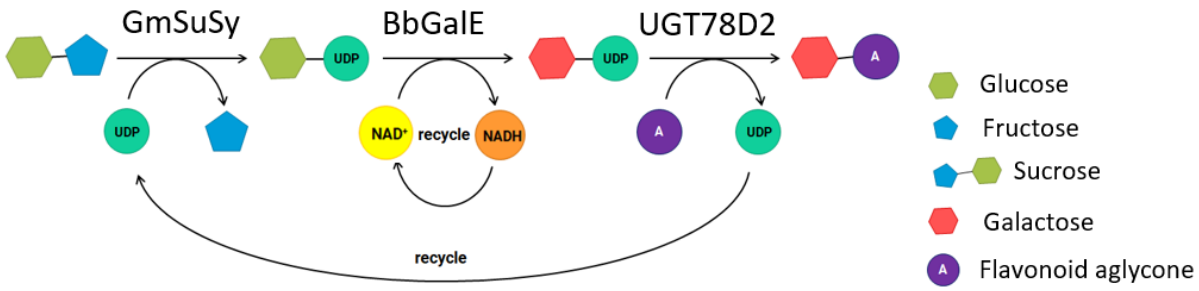

Figure 1. General reaction scheme of enzymatic cascade leading to flavonoid galactosides.

Herein we would like to present our recent progress in the incorporation of UDP-glucose 4-epimerase from *Bifidobacterium bifidum* (BbGalE) and selective galactosyltransferase from *Morella rubra* (UGT78D2) within the cascade with sucrose synthase from *Glycine max* (GmSuSy) in the production of flavonoid galactosides [3-4].

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P-19 Oliwia Rożnowska

Sustainable management of waste biomass through biotransformation into polyhydroxyalkanoates

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The waste biomass presents a significant challenge to the current economy, but it can be transformed into high-value products through biotransformation in biotechnological processes. Biopolymers produced in this way, such as polyhydroxyalkanoates (PHA), provide an alternative to commonly used plastic polymers and can also be applicable as drug carriers or raw materials to bio-based cosmetics components. Sustainable waste management aims to prolong the life cycle of these materials and minimize the generation of new waste, maximizing their potential.

Polyhydroxyalkanoates (PHAs) are large-molecule compounds classified as bacterial biopolymers. They are synthesized by various strains of microorganisms as well as genetically modified plants. During synthesis, they accumulate intracellularly within bacterial cytoplasm, forming characteristic granules that serve as a reserve material. Microorganisms utilize these granules in situations where there is a shortage of essential nutrients such as nitrogen, phosphorus, or sulfur, but an excess of carbon is present. PHA presents an attractive alternative to synthetic polymers due to their similar mechanical properties, biocompatibility, and ability to biodegradation. Despite their advantages, the production costs of biodegradable polymers remain high. However, by integrating waste resources into their synthesis, we can significantly reduce their production costs [1].

In industry, PHA commonly exists in several forms: medium-chain length (mcl-PHA), poly[(R)-3-hydroxybutyrate] (PHB), as well as copolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx). These compounds are synthesized by various bacterial strains including *Pseudomonas putida, Ralstonia eutropha, Alcaligenes latus, Escherichia coli*, and *Aeromonas hydrophila* [2]. *Pseudomonas putida* is a Gram-negative bacterial strain capable of producing mcl-PHA from carbon sources such as volatile fatty acids resulting from methane fermentation, fatty acids from plant extracts, as well as glucose, fructose, or glycerol [4-6]. Waste in the form of coffee grounds and post-extraction rapeseed meal poses a significant challenge in managing waste from the food industry sector due to their substantial quantity. The potential transformation of these wastes through biotransformation into useful high-quality products represents a promising pathway to increase their added value, in line with the principles of sustainable economy.

This study focuses on valorizing spent coffee grounds and post-extraction rapeseed byproduct, as a carbon sources for PHA synthesis using *Pseudomonas putida.* The research began with the utility of coffee oil as a carbon source for microorganisms to conduct the PHA biosynthesis process. To achieve this, the spent coffee grounds were spread on a tray and dried. Subsequently, they were subjected to extraction using a Soxhlet extractor with hexane as the solvent. The extractions were carried out for 2 hours. The obtained oil dissolved in

hexane was purified by evaporation using a rotary evaporator (temperature 60°C, 50 rpm) for approximately 15 minutes. The next stage of the research involved chromatographic analysis using LC-MS of the obtained oil to exclude potential bacterial growth inhibitors [6]. In another part of the experiment, acid hydrolysis (1% H_2SO_4, temperature 121°C) was performed for post-extraction rapeseed meal and coffee grounds, and the composition of the resulting hydrolysate was examined [4]. The research results represent the initial stages of developing technology for the utilization of bio-waste. The extraction efficiency from coffee grounds is promising. It is worth noting that the extraction process also generates post-extraction waste. The remaining material can be utilized as a potential carbon source, thus aiming for maximum waste utilization.

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P-20 Oliwia Rożnowska

Surface characterization of an inorganic membrane-enzyme system for environmental applications

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Currently, microplastic (MP) detection methods are getting more developed, although the sample preparation step becomes a bottleneck of the studies. This multi-step, time-consuming and expensive environmental sample preparation procedure for microplastic analysis requires simplification and standardization. For complex matrices like wastewater, a single sample preparation procedure takes approx. 17 days and require multiple sample treatments with different chemicals/reagents and several filtration steps. One of the most time-consuming steps is enzymatic treatments of the sample, to remove proteins and cellulose from the analysed material. Besides their length, taking at least 7 days, they also require additional sample filtrations, which carry the risk of microparticle loss during multiple repetitions of this procedure. Moreover, due to catalyst usage, this procedure is also expensive.

According to the literature, the use of membranes is the most efficient method of physical microparticle separation [1] whereas the enzymatic treatment of the sample allows impurities removal in mild conditions, which is especially important for further investigation of unaltered MPs. Based on this knowledge, new solutions in sample preparation procedures are still emerging to achieve better results in terms of the separation of a wide range of micro-sized polymers with appropriate shapes and sizes found in the environment. For this purpose, inorganic membranes were used to serve as matrixes for enzymes from various catalytic groups to form biologically active systems. Within the performed research advanced physicochemical as well as dispersion-morphological characterization and comparison of the membranes before and after immobilization were analyzed to understand their mechanism of action and properly design further steps of the sample preparation process.

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P-21 Aleksandra Rybak

Microbial enzymatic adaptations for biotransformations of selected API

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Aquatic organisms are constantly exposed to emerging contaminants from wastewater treatment plants (WWTPs). Chemotherapy medications: Methotrexate (MX), Vinorelbine (VINO), 5-Fluorouracil (5-FU), and Mycophenolic acid (MPA) – an immunosuppressant medication are concerning contaminants as their increasing use with the growing number of cancer patients and solid organ transplant recipients worldwide increases the potential risk to aquatic organisms exposed to wastewater discharges. Therefore, the main task of the presented study was a thorough analysis of the impact of the test compounds (MTX, 5-FU, VINO, MPA) at different concentrations on the growth of the microorganisms and their enzymatic activity.

Analyses were conducted for strains isolated from the artificial Lake Malta in Poznan. Individual strains were isolated from water samples and identified with the use of the matrix-assisted laser desorption/ionization (MALDI) technique. Further, the selected strains and concentrations of the test compounds were then used to establish cultures for enzymatic activity analyses. Samples were taken from the cultures at regular intervals, and proteins were isolated. Their concentrations were determined using the Bradford method and changes in the activity of selected enzymes (catalase, superoxide dismutase, S-glutathione transferase) were analyzed.

Agents such as Methotrexate, and 5-Fluorouracil belonging to the group of antimetabolites share structural similarities with compounds formed during normal cellular metabolism. Most antimetabolites have high cell cycle specificity and can target and arrest DNA replication in neoplastic cells[1]. Methotrexate is used in chemotherapy and as an immunosuppressant in auto-immune diseases. MTX may be dangerous if administered improperly. The most serious possible adverse effect is severe myelosuppression. This is responsible for the majority of the relatively rare deaths caused by MTX. Other side effects include bone marrow suppression, liver fibrosis, pneumonia, and baldness [2]. 5-Fluorouracil is still a common cancer drug. It has played an important role in the treatment of colon cancer since 1957 and is also used in patients with breast and other cancers, such as head and neck cancer [3]. Vinorelbine is a semi-synthetic vinca-alkaloid with a broad spectrum of anti-tumor activity. The mechanism of action is to interfere with the polymerization of tubulin. Tubulin is a protein responsible for building the microtubule system that forms during cell division. Among the alkaloid anticancer drugs, VINO was frequently detected in aquatic environments. These compounds have a high potential for persistence and bioaccumulation in sediments and organisms [4]. Mycophenolic acid is commonly used to prevent graft rejection in solid organ transplant recipients. The main concern with long-term use of immunosuppressive drugs is the risk of developing cancer [5, 6].

The effect of the tested compounds was different for each strain. Moreover, variations in bacterial enzymatic activity suggest various adaptation mechanisms for the metabolism of the tested compounds.

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P-22 Aleksandra Rybak

Influence of seasons on fresh-water bacterial community changes – a biochemical approach

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Community analysis is a valuable tool in environmental research, giving an opportunity to follow the effects of species cooperation and in the common effort of stress mitigation [1]. The seasons of the year are one of the factors occurring naturally, but due to the current climate changes, the extremes of each season are changing rapidly. This research shows the effect of seasons on the river water bacterial community in the neighborhood of the wastewater treatment plant discharge station.

To analyze microbial community changes, samples were taken every two months, starting from October 2023 to April 2024. The sampling points were chosen based on the location of the main Poznan WWTP discharge point: 1 km upstream (W-1) of the WWTP, directly at the WWTP discharge point (W0), and 1 km downstream (W+1) of the WWTP. During the sampling period, an abnormally high level of water was observed in February 2024 (above the warning level).

For the community analysis, the EcoPlates were used, to evaluate the functional diversity of bacterial communities by measuring their ability to oxidize carbon substrates. The EcoPlate is a 96-well microplate containing 31 common carbon sources from a total of five compound groups - carbohydrates, carboxylic and ketonic acids, amines and amides, amino acids, and polymers - plus a blank well and a control, all replicated three times to control for variation in inoculum density [2,3]. Based on the results, the Shannon diversity index was calculated and the studied consortia were compared in terms of their ability to metabolize selected compounds. The results of the analysis show bacterial shifts in time, depending on the sampling point, making visible also the impact of additional microorganisms from the WWTP discharge.

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Exploring Biotransformation of Phosphonates Using Cyanobacteria

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Biotransformation is a green, sustainable, and cost-effective process in which bacteria, yeast, fungi, plant tissues, cell extracts, and isolated enzymes can be used as biocatalysts [1]. The selection of suitable biocatalysts for specific reactions is a key stage of the process. In this study, we highlight the potential of cyanobacteria as an efficacious biotransformation platform for the synthesis of phosphonates. Cyanobacteria, photosynthetic prokaryotes, hold significant advantages, including minimal nutritional requirements; physiological resistance, metabolic plasticity, ease of genetic manipulation or tolerance to unfavorable conditions which makes them suitable for applications in biocatalysis [1, 2]. Cyanobacteria are known for their ability to reduce C=C bonds and carbonyl functional groups, however, their full spectrum of capabilities remains to be probed [3].

In this research, cultures of *Limnospira indica* PCC 8005*, Limnospira maxima* CCALA 27*, Leptolyngbya foveolarum* CCALA 76 and *Nodularia sphaerocarpa* CCALA 114 cultures were deployed as biocatalyst in a biotransformation of epoxyphosphonate (epoxymethyl dimethyl phosphonate). The procedure was executed over a period of 7 days at a stable temperature of 29 °C (±1) under continuous illumination and under stationary conditions. Products were extracted using ethyl acetate and subsequently analyzed by ^{31}P NMR.

After 24 h of the process *L. indica* and *L. maxima* efficiently transformed epoxyphosphonate with a conversion degree exceeding 99%. By contrast, to achieve high conversion rates in reactions mediated by *L. foveolarum* and *N. sphaerocarpa* (96% and 76% respectively), it was indispensable to prolong the biotransformation duration to 7 days. To evaluate the potential toxic impact of phosphonates on cyanobacteria, minimum inhibitory concentrations were determined using AlamarBlue Cell Viability Assay.

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P-24 Sandra Sordon

Exploring the Potential of Flavin-Dependent Halogenases in the Modification of Flavonoids

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Flavonoids are a large group of secondary metabolites widely distributed in plants. They exert numerous important physiological functions in plants, having considerable influence on growth and development of plants, protect them from UV radiation, bacterial and fungal infections and provide color to fruits and flowers [1]. Flavonoids found in food have beneficial effects on human health. They exhibit a diverse spectrum of biological activities such as: antioxidant, antitumor, anti-inflammatory, antiviral, estrogenic and anticancer [2]. Introducing halogens into structures of aromatic natural products could enhance their biological activities and physicochemical properties [3], however data on the biological activity of halogenated flavonoids is scant due to very limited occurrence in nature and difficulty in preparation. Despite the existence of numerous chemical halogenation methods, they often require potentially hazardous reagents, high temperatures and usually, they are characterized by low regio- and stereoselectivity, which is their biggest drawback [4]. Recently, there has been a growing interest in flavin-dependent halogenases (FDHs) as promising biocatalysts that allow for the insertion of halogen atoms into the structures of various compounds with high selectivity and efficiency including those positions in a structure that are electronically unfavorable. Flavin-dependent halogenases (FDH) utilize only oxygen from air, the $FADH_2$ as a cofactor, and halide salts. FDH catalysis occurs in water-based solutions at ambient temperature [5].

In this study, flavin-dependent halogenases: Rdc2 [6] and RadH (with confirmed activity for flavonoids) [7] were employed in the biotransformation process to determine their substrate specificity for a broad panel of flavonoid substrates (various classes of flavonoids), as well as to optimize biotransformation process in order to obtain halogenation products on a preparative scale.

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P-25 Daria Szada

An improved enzymatic approach to the synthesis of (*S*)-ibuprofen; a dynamic kinetic resolution of an ibuprofen methyl ester racemic mixture

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In recent years, the use of biocatalysis has increased rapidly in many industries. A particular application of enzymatic catalysis is in the rapidly growing pharmaceutical industry since biocatalysts are used to synthesize a variety of important pharmaceutically active substances (API). [1]. Several advantages make enzymes effective as biocatalysts for chemical reactions. Firstly, enzyme-catalyzed reactions are characterized by high chemo-, regio-, and stereoselectivity, which can be difficult to achieve with traditional synthesis methods, and is of great significance in the production of APIs. In addition, enzyme catalysts are capable of conducting reactions under mild conditions of temperature, pH, or pressure, thereby reducing process costs. In most cases, enzymatic reactions can be carried out in an aqueous environment, which reduces the risk associated with organic solvents. Furthermore, enzymes can be easily isolated from renewable sources, are biodegradable, non-toxic, and are consistent with Green Chemistry principles [2,3]. Research into enzyme catalysts continues to improve their efficiency to make them even more effective. The immobilization of enzymes improves chemical synthesis processes since it enhances the stability of the enzyme as well as facilitates the separation of the catalyst from the reaction medium and its reuse [4].

Ibuprofen has been widely used throughout the world for many years. Ibuprofen belongs to the nonsteroidal anti-inflammatory drug (NSAID) class. The substance has anti-inflammatory, analgesic, as well as antipyretic effects. Commercially, ibuprofen is mainly available as a racemic mixture of (R)-ibuprofen and (S)-ibuprofen. Nevertheless, it is important to note that both enantiomers possess different pharmacological properties. The pharmacological activity of ibuprofen is mainly manifested by (S)-ibuprofen, which is up to 160 times more active than (R)-ibuprofen. (S)-ibuprofen can inhibit both cyclooxygenase-1 and cyclooxygenase-2 to an equal degree, while the (R)-enantiomer is much weaker in inhibiting COX-1 and COX-2. The dissimilarity of the two enantiomeric forms is also manifested in their metabolic profile, as (R)-ibuprofen becomes involved in lipid metabolism pathways, which is not the case with the (S)-enantiomer of ibuprofen [5,6]. In traditional synthesis methods, ibuprofen is primarily produced as a racemic mixture. The use of enzymatic methods makes it possible to obtain predominantly one, more desirable enantiomer of ibuprofen.

This study aimed to demonstrate the utility of immobilized lipase from *Candida rugosa* as a biocatalyst for the separation reaction of a racemic mixture of ibuprofen methyl ester, ultimately leading to (S)-ibuprofen as the desired product. During the conducted studies, the effect of reaction time was examined, the significance of the type of organic solvent was evaluated, and an attempt was made to demonstrate the differences in the case of changing pH and adding DMSO (kinetic/dynamic kinetic resolution) on the efficiency of biocatalytic conversion. It was shown that the studied parameters have a significant effect on the

conversion of the substrate and, on the enantiomeric excess of the obtained (S)-ibuprofen over the (R)-ibuprofen. The results indicated that isooctane was a more suitable organic solvent, as substrate conversion was higher in its presence than in hexane (for the process conducted at pH = 7, a conversion rate of 48% was obtained for the sample in isooctane and 36% for the sample in hexane). To evaluate the effect of time on enzymatic reaction, samples were taken for testing every 48 hours, and the most favorable results were obtained after 144 hours. As a result of increasing the pH of the reaction and adding DMSO, dynamic kinetic resolution of ibuprofen methyl ester was enabled, and in situ racemization of the unconverted (R)-ester to the (S)-ester was carried out, resulting in a higher conversion rate. For the process carried out in isooctane, the use of NaHCO3-NaOH buffer at pH = 9.5 instead of phosphate buffer at pH = 7 allowed an increase in the substrate conversion rate from 48% to 83%. Moreover, the addition of DMSO made it possible to increase the conversion of ibuprofen methyl ester to 95%.

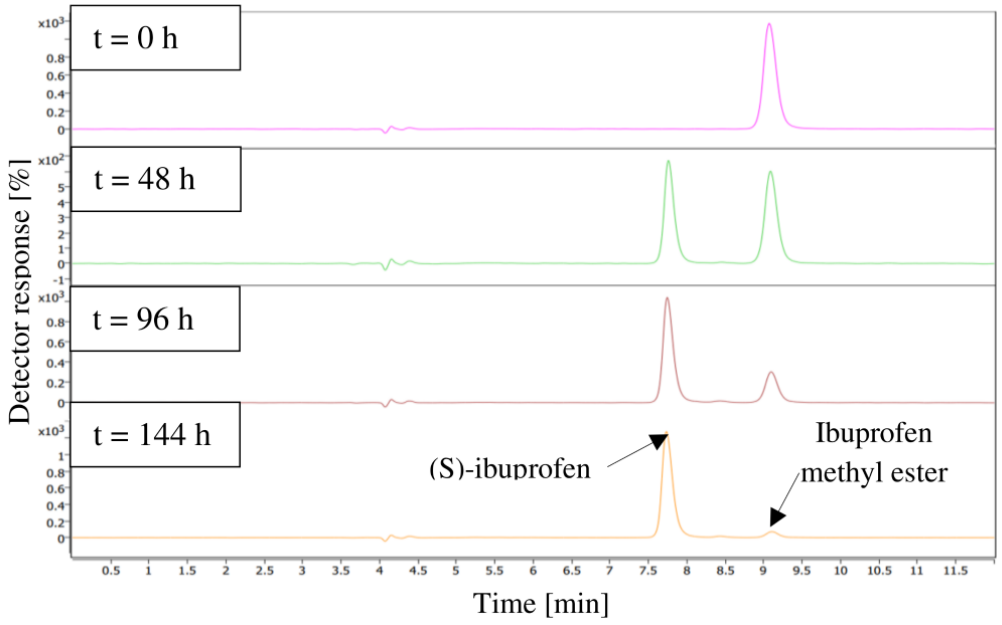

Figure 1. Chromatograms of the process carried out in isooctane and $NaCO_3$-NaOH at pH = 9,5 in the presence of DMSO.

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P-26 Maciej Szaleniec

The Initiation Phase of the Catalytic Cycle in the Glycyl-Radical Enzyme Benzylsuccinate Synthase – Modelling and Experiment

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Benzylsuccinate synthase (BSS) belongs to the family of fumarate-adding enzymes which is itself part of the growing superfamily of glycyl radical enzymes (GRE)[1]. GRE are involved in surprisingly different, but always chemically demanding reactions in anaerobic metabolic pathways of Bacteria, Archaea, and Eukarya. In addition to fumarate-adding enzymes, the currently known families of GRE consist of the pyruvate formate lyases (PFL), type III anaerobic ribonucleotide reductases (ARNR), glycerol or diol dehydratases, hydroxyproline dehydratases, arylacetate decarboxylases, choline or isethionate lyases and phosphonate-cleaving C-P lyases.

The BSS enzyme catalyzes the radical-based addition of toluene to a fumarate cosubstrate. The process is initiated by hydrogen transfer from a conserved cysteine (Cys493) to the nearby glycyl radical (Gly829) in the active center of the enzyme (Fig. 1). Although the BSS mechanism is studied for many years, this first step of the reaction was never properly examined in the context of the already known structure of the enzyme.

In this poster we analyze this step by comprehensive QM:MM modeling, predicting (i) the influence of bound substrates or products, (ii) the energy profiles of forward- and backward hydrogen-transfer reactions, (iii) their kinetic constants and potential mechanisms, (iv) enantiospecificity differences and (v) kinetic isotope effects. We also used microkinetic constants derived from calculations to estimate overall rates of H/D transfer and to propose potential mechanisms of H/D exchange at radical Gly829 [2].

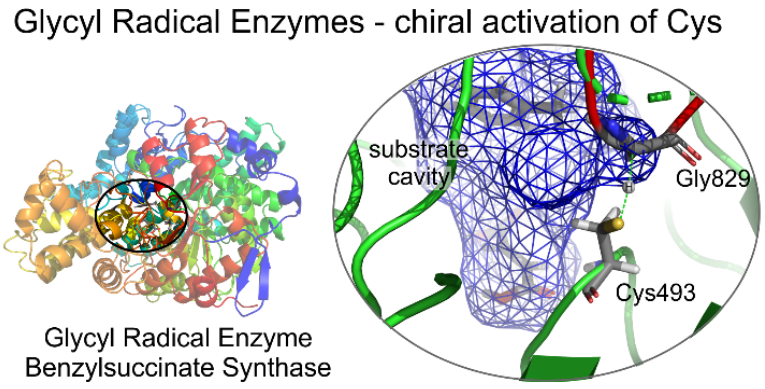

Fig. 1. Catalytic subunit of BSS (left) and a close-up of the BSS active site with substrate cavity holding fumarate and toluene delineated by a blue mesh.

We supported some computational predictions experimentally, providing evidence for predicted H/D-exchange reactions into the product and at the glycyl radical site. We have conducted kinetic experiments analyzing the H/D exchange rate of substrates and products when the reaction was conducted with deuterated substrates or in D_2O. We have also reanalyzed the H/D exchange of the glycyl radical in BSS using EPR spectra after changing the solvent to D_2O and back again to H_2O [3].

Our data indicate that the hydrogen transfer reactions between the active site glycyl and cysteine are principally reversible, but their rates differ strongly depending on their stereochemical orientation, transfer of protium or deuterium, and the presence or absence of substrates or products in the active site. This is particularly evident for the isotope exchange reaction of the remaining protium atom of the glycyl radical to deuterium, which appears to be dependent on substrate or product binding, explaining why it has been observed in some, but not all glycyl-radical enzymes.

Our results show that the radical on Gly829 requires bound substrates or product to enable H-transfer or H/D exchange with Cys493, while these reactions are precluded in apo-BSS. Moreover, the enzyme retains the H- or D-atoms of the glycyl radicals when acting with the preferred stereospecificity but is able to initiate H/D exchange into the glycyl radical at a significantly slower rate (and with even slower reverse D/H exchange) by occasionally reacting in the non-preferential stereospecificity. Such behavior of the BSS enzyme may be valid for all GRE, indicating that substrate or product binding may be a prerequisite for both initiating H-transfer between the active site Gly and Cys residues and the reactions involved in the H/D exchange of the glycyl radical.

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Acknowledgments

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P-27 Marzena Szawara

Searching for selective and safe inhibitors of epoxide hydrolase of juvenile hormone targeting selected pests

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Maintaining biodiversity requires pollinating insects. Worldwide reports of pollinator losses in recent years have major ecological and economic repercussions. Deterioration and loss of habitat are two factors that have a negative impact on the population of these insects. The use of plant protection products by humans, whose primary objective is to lower the pesticides among crops is however, a reason that is becoming more and more prevalent.

We think it's possible to maintain pollinators while also managing insect numbers. It is already known, that JHEH, and enzyme which is crucial for insects' metamorphosis, can be used as a target for insect's control. However, so far developed pesticides were unspecific. In our research we are combining computational and experimental methods used for drug design, to propose highly selective compounds, for selective pests' control.

We aim to explore a unique approach which is based on intramolecular voids analysis and molecular dynamics simulations (MD) in co-solvents which will guide pharmacophore design. Identified differences and similarities between different insect JHEH will help in the design of new pharmacophores and subsequently species-specific inhibitors.

Ultimately, our goal is to conduct an extensive study of selected recombinant proteins from ten organisms to best replicate the taxonomic cross-section of species and experimentally confirm the results of the computational analysis carried out.

Acknowledgements
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Microbial synthesis of sesamol from piperonylic acid

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Sesamol is an organic compound (derivative of phenol) with wide spectrum important biological activities such as antifungal, anti-inflammatory, antidepressant, neuroprotective, wound healing, and anti-aging [1]. Moreover, it is useful as an antioxidant in edible fats. Currently, sesamol is obtained by extraction from sesame oil or by chemical synthesis from piperonal [2].

This research presents the novel method of obtaining sesamol from piperonylic acid using a biotechnological route. Accordingly to our studies, *Monascus* strains exhibit ability to conduct the biotransormation of piperonylic acid to this highly demanded compound, which constitutes an attractive alternative to synthetic methods. The biotransformation conducted in the prep-scale process (1g/L) led to obtaining sesamol with the yield 36.7%. Additionally, a biological activity of nano-conjugate of sesamol and zinc oxide nanoparticles (ZnONPs) was studied against human dermal fibroblast cell lines.

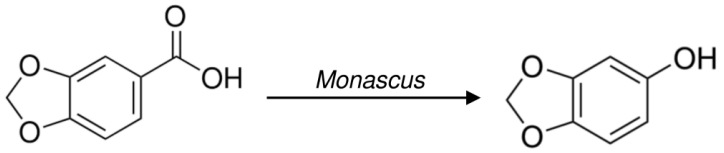

Figure 1. Biotranformation of piperonylic acid to sesamol.

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Acknowledgments

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P-29 Mateusz Tataruch

Thermal inactivation of KSTD1 from *Rhodococcus erythropolis* – preliminary studies

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Steroids are important synthons of many drugs and compounds from the "fine chemicals" group. The wide range of steroid uses results in the continuous development of methods for obtaining these compounds. The challenge is to search for effective enzymatic tools for the green synthesis of this type of compounds and to improve the bioprocesses catalyzed by them. This is based on deepening knowledge about their stabilization and understanding the mechanisms of their inactivation.

3-Ketosteroid delta1-dehydrogenase (KSTD1, PDB ID: 4C3Y) from *Rhodococcus erythropolis* is an FAD-dependent enzyme that catalyses dehydrogenation between C1 and C2 atoms of the steroid ring A of 3-ketosteroid substrates. This enzyme is a high-class biotool for one-step, selective synthesis of steroid compounds, especially desired in the pharmacy industry. The enzyme contains a FAD-binding and catalytic domain, between which the active site is situated, and it is determined to be monomeric in solution. [1,2,3]

The thermal stability of KSTD1 was investigated under storage conditions without stabilizers at temperature range from 25 to 50 °C. Inactivation data were analyzed to assess compliance with the “one step – two steps” mechanism using isotemperature evaluation. Discontinuity observed on the Arrhenius plot indicates that the thermal inactivation of KSTD1 follows a more complex mechanism. Moreover, the influence of potential stabilizing compounds such as glucose, ketosteroid substrate (androst-4-en-3,17-dione) and artificial electron acceptor (2,6-dichloroindophenol) on improving enzyme stability was separately examined.

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Enzymatic valorization of lignocellulosic biomass – The influence of deep eutectic solvents and ionic liquids on the activity of cellulolytic enzymes

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The valorization of lignocellulosic biomass has become one of the most frequently discussed topic both in industry and science. Plant waste is not only a renewable source of energy, but also an accessible and affordable source of polysaccharides, which can be transformed to valuable chemicals (e.g. levulinic acid, furfural, glucose) useful in pharmaceutical, food, and fuel industries. Furthermore, the methods for biomass processing are constantly being improved to reduce their impact on natural environment and simultaneously increase their efficiency. One of these methods includes applying cellulolytic enzymes for biomass hydrolysis to monosaccharides. However, one of the main lignocellulose components, lignin, acts as a mechanical barrier for biocatalysts, negatively affecting the processing of cellulose and hemicelluloses [1]. Additionally, lignin can bind non-covalently to applied cellulase, which further reduces the efficiency of hydrolysis [2]. Because of that, it is recommended to pretreat biomass before enzymatic processing, in order to remove part of lignin from the material. One of the methods for such a process is applying deep eutectic solvents (DESs), which are non-toxic, have low volatility, and have low environmental impact [3]. Additionally, they have an excellent ability to dissolve lignin without impact on other lignocellulose components [4]. However, certain DESs might deactivate cellulases, necessitating the removal of the solvent from the material prior to enzymatic hydrolysis. To circumvent this requirement, enzymes exhibiting strong resistance to DESs are recommended for use. While literature does provide examples of biocatalysts with stable activity in the presence of DESs, information regarding cellulolytic enzymes is currently limited [5,6].

In this work, the impact of chosen DESs on the enzymatic activity of cellulases from *Aspergillus niger*, *Trichoderma reesei*, and cellulolytic preparation Viscozyme L is presented. It came out that all of the applied solvents affect negatively the activity of biocatalysts, especially those containing acids (lactic acid and levulinic acid), which excludes the one-pot process of biomass transformation with cellulases and DESs. The experiments showed that Viscozyme L is the most resistant to DESs [7].

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P-31 Katarzyna Wińska

New hydroxylactones obtained by biotransformation of bicyclic halolactones with three methyl groups and their antimicrobial activity

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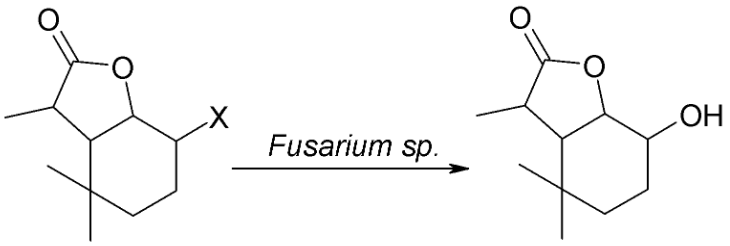
Lactones are a large group of naturally occurring compounds. They are characterized by a variety of biological activities [1-2]. Of particular interest are lactones containing halogen atoms in their structure. Synthetic halolactones show antiproliferative activity [3], antimicrobial activity, cytotoxic activity [4], and the ability to inhibit photosynthesis [5]. In turn, hydroxylactones, which are often isolated from natural sources, show antimicrobial [6-7], fungistatic [8], cytotoxic [9] activity.



X = Cl, Br or I

During our research, halolactones with a gem-dimethylcyclohexane system in the cyclohexane ring and a methyl group in the lactone ring were obtained by chemical synthesis. Halolactones occurring as mixtures of two diasteroisomers were biotransformed using strains of the genus Fusarium, yielding the corresponding hydroxylactones. Knowing that both halo- and hydroxylactones can exhibit antimicrobial activity, compounds obtained by both chemical synthesis and biotransformation were subjected to such tests.

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Carvone biotransformations in carrot cell cultures

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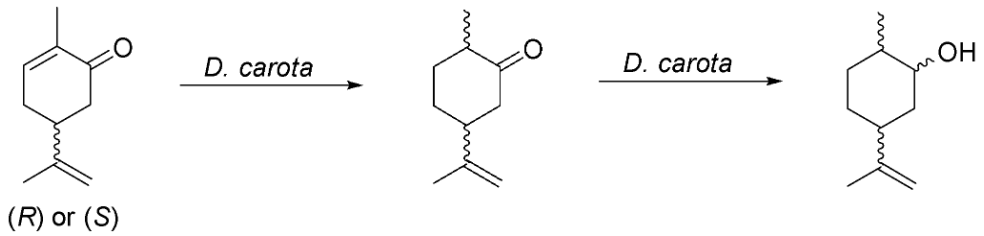
Plant cell cultures have been shown to be effective at performing bioconversion or biotransformation reactions on various organic compounds, including the conversion of ketones into optically pure hydroxy compounds [1]. Several studies have demonstrated that plant cell cultures from different species, such as carrot, tobacco and gardenia [2], can biotransform ketones into the corresponding alcohols [3,4].



Carvone enantiomers - (4R)-(-)-carvone and (4S)-(+)-carvone were substrates in the biotransformation carried out using carrot cell culture. To obtain the starting material, which was carrot callus, two different combinations of growth regulators were used - NAA 2 mg/l, BA 0.2 mg/l and BA 2.0 mg/l, NAA 0.2 mg/l. Biotransformation were carried out for three weeks. It was observed that in the first step the double bond in the cyclohexane ring was reduced and then the carbonyl group was reduced. The final biotransformation products were the corresponding dihydrocarveols.

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P-33 Agnieszka Wojtkiewicz

Bioinformatic analysis of 3-ketosteroid dehydrogenases family: in between the sequence & substrate specificity

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3-Ketosteroid dehydrogenases (KstDs) are crucial in steroid biotransformation, offering diverse applications across pharmaceutical, biotechnological, environmental, and medical sectors [1]. Due to the ubiquity and diversity of steroid compounds, bacteria have evolved strategies to modify and degrade these compounds in various niches [2]. Consequently, KstDs, key enzymes in steroid degradation, are expressed in numerous variants that differ in structure, biochemical properties, and, most importantly from an application standpoint, substrate specificity[3]. We analyzed over 300 KstD sequences similar to three selected KstDs: AcmB2[4], KstD1 [5], and KstD4 [6]. (Fig. 1).

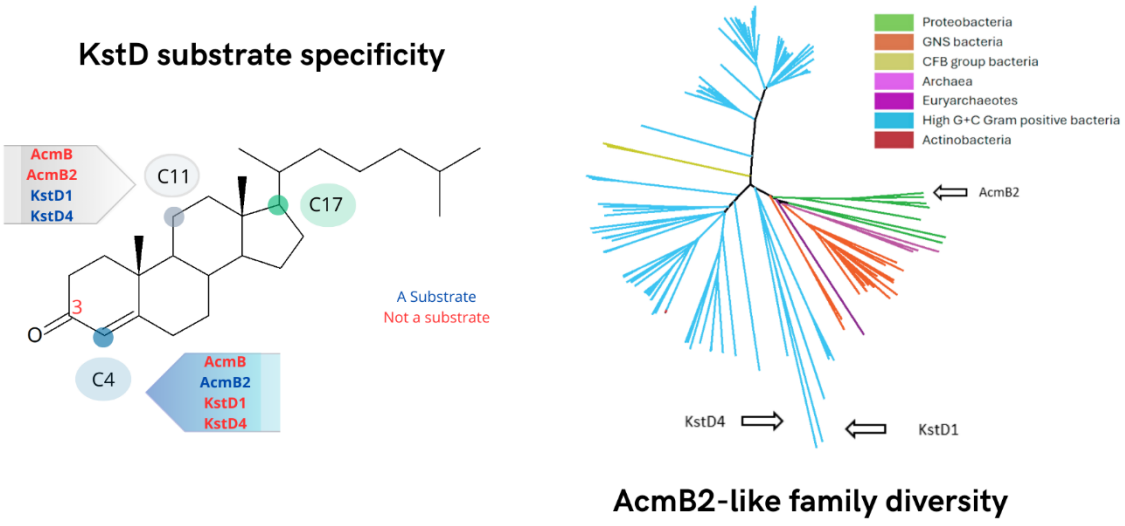

Figure 1. The substrate specificity of selected KstDs: AcmB2 from *Sterolibacterium denitrificans*, KstD4 from *Arthrobacter simplex*, and KstD1 from *Rhodococcus erythropolis.* The structure of cholest-4-en-3-one highlights positions where substituents are allowed (blue) or forbidden (red) to observe 1,2-dehydrogenation activity. Additionally, the diversity of the AcmB-like family is presented as a phylogenetic tree of amino acid sequences, colored by the type of bacteria.

In our work we prepared the analysis for three groups of KstDs: AcmB2 from anaerobic *Sterolibacterium denitrificans*, KstD4 from obligate aerobic *Arthrobacter simplex*, and KstD1 from aerobic *Rhodococcus erythropolis.* The conducted analysis not only identified the type of bacteria producing a specific KstD variant but also determined the habitat of these bacteria and their environmental conditions, whether aerobic or anaerobic. Identifying this latter parameter provides insights into the prevalence of anaerobic bacteria, which have a distinct steroid degradation pathway compared to aerobic bacteria. Such studies can contribute to proposing new candidates for isolation from the environment, with the aim of

discovering new variants of steroid degradation enzymes in addition to KstD, as well as potentially identifying KstDs with specific substrate specificity.

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P-34 Katharina Müller

Investigating the mechanism of acetophenone carboxylase

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Aromatic ketones such as acetone and acetophenone are common intermediates of natural secondary metabolism. As they are chemically inert, these substrates must be activated prior to microbial degradation. Ketone activation proceeds either by oxygenation or by carboxylation [1,2].

Acetone carboxylase (Acx) and acetophenone carboxylase (Apc) first activate both of their substrates (*i.e.*, the ketone and HCO_3^-) by phosphorylation [1]. In Acx, both substrates are phosphorylated at the same activation site. Then, the activated substrates are transferred over a distance of 40 Å from the nucleotide binding site to the catalytic site via an internal channel [3]. In contrast, substrate phosphorylation occurs at two distinct sites in Apc. Phosphorylation sites are 50 Å apart from each other, which precludes direct interaction of the activated substrates. Free diffusion between the activation sites is not possible either as the activated substrates are readily decomposed in water. The Apc core complex ($[\alpha\alpha'\beta\gamma]_2$) moreover lacks internal substrate channels for acetophenone-*enol*-phosphate transport [2]. However, in contrast to Acx, Apc possesses an additional Apcε subunit, which is not part of the Apc complex, but is indispensable for acetophenone carboxylation [4]. The actual function of Apcε is still at issue. It has been hypothesized that Apcε forms a lid which shields the activated substrates from water and allows them to diffuse to the catalytic subunit or induces conformational changes in Apc to bring the substrate binding sites into close proximity [2].

Here, we study the specificity of interactions between Apcε and the core complex. We generate *Aromatoleum spp. ΔapcE* deletion mutants and complement them with heterologous Apcε subunits of high or low sequence identity (*e.g.*, Apcε from *Aromatoleum spp*. or *Nevskia ramosa*). Comparing the growth of these mutants on acetophenone, we evaluate which level of interaction specificity between Apcε and core complex is required to sustain enzyme activity.

References

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