Wrocław University of Environmental and Life Sciences

Faculty of Biotechnology and Food Science

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Biotechnology – Research and Industrial Applications

20–21.06.2018
Wrocław – POLAND

UWP

BIOTECHNOLOGY – RESEARCH AND INDUSTRIAL APPLICATIONS

20–21.06.2018

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TIMETABLE

WEDNESDAY 20.06.2018 Scientific-Didactic Center of Wrocław University of Environmental and Life Sciences, Grunwaldzki Square 24a, 50-365 Wrocław	
7.30–17.00	**REGISTRATION**
9.00–9.15	**Opening Ceremony and Welcome, Jan Paweł II Hall** **Prof. Agnieszka Kita – Vice-Dean for Research and Development** **Prof. Tadeusz Trziszka – Rector of Wrocław University of Environmental and Life Sciences**
9.15–10.00	**Plenary Lecture – Engineering oleaginous yeast *Yarrowia lipolytica* for production of high-value metabolite**, *Irina Borodina, Technical University of Denmark*
Session 1.	**MICROORGANISMS**
	Chair: prof. Małgorzata Robak, prof. Andriy Sibirny
10.00–11.00	**Plenary Lectures: *Yarrowia*, a reservoir of promising oleaginous yeasts for biotechnological applications**, *Cécile Neuvéglise, INRA Jouy-en-Josas, France* **Production of organic acids by the yeast *Yarrowia lipolytica***, *Stephan Mauersberger, Andreas Aurich, Technische Universität Dresden, Germany*
11.00–11.30	Short Communications (1.1–1.2)
11.30–12.00	Coffee break
12.00–13.30	Short Communications (1.3–1.6)
13.30–14.30	Lunch
Session 2.	**BIOSYNTHESIS, BIODEGRADATIONS & BIOTRANFORMATIONS**
	Chair: dr hab. Anna Gliszczyńska, prof. Waldemar Rymowicz
14.30–15.30	**Plenary Lectures:** **Thermotolerant yeast *Ogataea polymorpha* as promising organism for conversion of lignocellulosics and by-product glycerol to ethanol**, *Andriy Sibirny et al., NAS of Ukraine, Ukraine, University of Rzeszów, Poland* **Formation of C-C bond and chiral amines using biocatalysis** *Wolfgang Kroutil et al., University of Graz, Austria*
15.30–16.15	Short Communications (2.1–2.3)
16.15	Coffee break
16.15–17.15	Poster session
19.00	Dinner restaurant Dwór Polski, Market Square 5

THURSDAY 21.06.2018	
Session 3.	**PLANT & ALGAL BIOTECHNOLOGY**
	Chair: dr Stephan Mauersberger, dr Xymena Połomska
9.00–9.30	**Plenary Lecture: Green Bioprinting – A tool for creating green 3D-cell/matrix**, *Felix Krujatz et al., TU Dresden, Institute of Natural Materials Technology, Dresden*
9.30–10.30	Short Communications (3.1–3.4)
10.30–10.45	Coffee break
Session 4.	**ENZYMES & PEPTIDES**
	Chair: prof. Cécile Neuvéglise, prof. Józefa Chrzanowska
10.45–11.45	**Plenary Lectures:** **Surface display of proteins in yeast-from understanding basic concepts of cell wall biosynthesis to cell surface engineering**, *Vladimir Mrša et al., University of Zagreb, Croatia* **Lactoferrin and yolkin-derived proteins increase the proteolytic capacity of the serine protease cathepsin G important for an immune response** *Timo Burster et al., Nazarbayev University, Kazakhstan*
11.45–12.45	Short Communications (4.1–4.4)
	Closing of the conference
13.15–14.15	Lunch
14.15–17.00	City sightseeing (the most beautiful monuments of the capital of Lower Silesia – walk around the center of Wrocław with a guide from 15.00 to 17.00, the Old Town, Ostrów Tumski and the Old Town Promenade, St. Elizabeth's Church, St. Mary Magdalene's Church, legends of Wrocław etc.)

CONTENTS

SYMPOSIUM CONCERNING YOLKIN ON 19.06.2018 OPENING INTERNATIONAL SCIENTIFIC CONFERENCE

PLENARY LECTURES

Irina Borodina

ENGINEERING OLEAGINOUS YEAST *YARROWIA LIPOLYTICA* FOR PRODUCTION OF HIGH-VALUE METABOLITES

The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark
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One of the major applications of synthetic biology is development of novel cell factories for sustainable production of bulk and specialty chemicals. The recent advances in CRISPR-based genome editing of yeast made construction of yeast cell factories cheaper and faster. These genetic tools facilitate iterative cycles of metabolic engineering, where the cellular metabolism is systematically re-wired towards higher titer, rate and yield of the target product(s). Oleaginous yeast *Yarrowia lipolytica* recently emerged as a work horse for production of acetyl-CoA and fatty acid derived metabolites. I will present examples of engineering *Y. lipolytica* for production of adipic acid, carotenoid feed additives, and insect pheromones for environmentally friendly pest control.

Cécile Neuvéglise

YARROWIA, A RESERVOIR OF PROMISING OLEAGINOUS YEASTS FOR BIOTECHNOLOGICAL APPLICATIONS

INRA Jouy-en-Josas, France

Yeasts have been exploited for decades for biotechnological applications. With the recent development of tools in the fields of genomics, metabolic engineering, and system/synthetic biology, *Yarrowia lipolytica* is becoming one of the most studied yeast species of biotechnological interest. Surprisingly, modified strains derive from a limited number of wild parental strains, including the French W29, the German H222 or the Polish A101. We thus investigated the biodiversity of strains at different taxonomic levels. First, we studied *Y. lipolytica* populations for phenotypic traits and genomic polymorphism. The ability of 58 strains to assimilate various substrates appeared very conserved. In contrast, the capacities of lipid synthesis and lipid accumulation were much more variable. Genomic analysis revealed a huge conservation of the genes, but some rearrangements in the chromosomal structure. As no clear correlations between genotypes and phenotypes were observed, especially for lipid metabolism, we investigated the biodiversity of different species of the *Yarrowia* clade. Surprisingly, whereas the phenotyping revealed no major differences, the genomic study held many surprises with genome sizes varying from 10.6 to 31.2 Mb. How genomic data were taken into account to study the metabolism of these yeasts will be discussed.

Stephan Mauersberger[1], Andreas Aurich[2]

PRODUCTION OF ORGANIC ACIDS BY THE YEAST *YARROWIA LIPOLYTICA*

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Functionalized carboxylic acids are highly versatile chemical species with a wide range of applications (e.g. as co-polymers, building blocks, acidulants). Therefore they are of special interest as biotechnologically available targets. The yeast *Yarrowia lipolytica* secretes high amounts (from 100 up to 200 g/L) of various organic acids, like citric (CA), isocitric (ICA), α-ketoglutaric (KGA) and pyruvic (PA) acids, under different conditions of growth limitation (by nitrogen or thiamine) from a carbon source excess. The aim of these studies was the application of genetically engineered *Y. lipolytica* strains in combination with the development of bioprocess fundamentals for the efficient production of TCA cycle and related acids (CA, ICA, KGA, PA) regarding the variable use of renewable substrates and industrial by-products (including plant oils, sucrose, biodiesel – FAME, and raw glycerol) [1, 2].

Depending on the carbon source, wild-type *Y. lipolytica* strains produce triggered by N-limitation, a characteristic CA/ICA ratio, on carbohydrates or glycerol of 90:10 and on plant oils (sunflower or rapeseed oil) or n-alkanes of 50:50 to 60:40. To examine, whether this CA/ICA product ratio can be influenced, isocitrate lyase (*ICL1*), aconitase (*ACO1, ACO2*), NADP- (*IDP1*) or NAD- (*IDH1*, *IDH2*) isocitrate dehydrogenases gene-dose-based overexpressing recombinant strains were constructed (using integrative multicopy vectors) containing multiple copies of these genes alone or combinations of them.

In the *ICL1* overexpressing recombinant strains the part of ICA on the whole product (CA + ICA) decreased to 3–7% on all tested carbon sources including plant oils and sucrose, significantly reducing the undesired ICA for CA production [3].

In contrast, the *ACO1* [4] (not the *ACO2*) and interestingly also the *IDP1* overexpression and a combination of them resulted in a product pattern shift in direction of ICA, reducing the undesired CA for ICA production. On sunflower oil the ICA proportion increased from 35–55% to 65–72% of total acid produced in shaking flasks experiments. Strains with increased copy numbers of both *ACO1* and *IDP1* showed the highest ICA selectivity up to maximally 75–85% in bioreactor experiments. By using wild-type or engineered *Y. lipolytica* strains the enantiomerically pure form of D-*threo*-isocitric acid (ICA), currently available as a speciality compound, can be produced now in large amounts (>105 g/L) and used as a building block for multigram organic synthesis, e.g. for clinically useful HIV protease inhibitors [5, 6].

Under conditions of controlled thiamine limitation (0.1–3.0 µg/L) the yeast *Y. lipolytica* is able to accumulate KGA and PA. The highest KGA concentrations (up to 115 g/L) and selectivities (>96%) were achieved with rapeseed oil using the strain *Y. lipolytica* H355. PA was produced up to 64 g/L and 80% selectivity from raw glycerol by the same strain. To develop a biotechnological process of KGA production by *Y. lipolytica* from raw glycerol, H355 derived recombinant strains (*IDP1*, *FUM1* – fumarese, *PYC1* – pyruvate carboxylase gene-dose-dependent overexpression) were applied to increase the KGA productivity (up to 186 g/L) and to reduce the amounts of by-products to 2–5%, e.g. PA as major by-product and fumarate, malate and succinate as minor by-products [7, 8].

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Andriy Sibirny[1,2], Olena Kurylenko[1], Justyna Ruchała[2], Marta Semkiv[1], Iwona Kata[2], Kostyantyn Dmytruk[1]

THERMOTOLERANT YEAST *OGATAEA POLYMORPHA* AS PROMISING ORGANISM FOR CONVERSION OF LIGNOCELLULOSICS AND BY-PRODUCT GLYCEROL TO ETHANOL

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Methylotrophic yeast *Ogataea (Hansenula) polymorpha* belongs to the most thermotolerant yeast organisms known with maximal growth temperature 50°C. Thermotolerance is important trait for alcoholic fermentation as process goes faster and energy could be saved due to smaller differences between fermentation and distillation temperatures. Thermotolerance could be especially important during 2nd generation ethanol production as allows using cellulases and hemicellulases (optimum temperature at 50°C and more) in the same vessel with thermotolerant yeast cells which convert sugars liberated by the enzymes to ethanol. This process is known as simultaneous saccharification and fermentation or SSF. *O. polymorpha* has additional promising features for 2nd generation ethanol production due to its ability to ferment important sugars of lignocellulosics as glucose, cellobiose and xylose and to grow on glycerol as the sole carbon and energy source. However, efficiencies of xylose alcoholic fermentation and glycerol conversion to ethanol by the wild-type strain of *O. polymorpha* are very low. Using combination of metabolic engineering and classical selection, ethanol production from xylose was increased 30–40 times and reached 15–17 g of ethanol/L at 45°C. Several new approached of metabolic engineering were developed and used. They include knock out of transcription activator *CAT8* and overexpression of genes *DAS1* and *TAL2* coding for peroxisomal transketolase (dihydroxyacetone synthase) and transaldolase, respectively. It was also found that knock out of *PEX3* gene involved in peroxisome biogenesis, similarly to knock out of DAS1 and TAL2, genes, practically totally blocked ethanol production from xylose (but not from glucose) though did not affect growth on xylose as sole carbon and energy source. Overexpression of TKL1 and TAL1 genes coding cytosolic transketolase and transaldolase, respectively, also increased ethanol production form xylose whereas knock out of these genes hampered growth on xylose with moderate effects on xylose alcoholic fermentation. New approach in classical selection was based on isolation of the mutants resistant to glycolysis inhibitor, anticancer drug 3-bromopyruvate. It was found that near 70% of 3-brompyruvate-resistant mutants are characterized by increase in ethanol production from xylose. Ethanol production from glycerol was improved due to overexpression of genes coding enzymes of the initial (GCY1, DAK1, GUT1 and GPD1, encoding glycerol dehydrogenase, dihydroxyacetone kinase, glycerol kinase and glycerol-3-phosphate dehydrogenase, respectively) and of the final steps of glycerol conversion to ethanol (PDC1 and ADH1 coding for pyruvate decarboxylase and alcohol dehydrogenase, respectively). Perspectives of further improvements of O. polymorpha for 2nd generation ethanol production are discussed.

Wolfgang Kroutil[1,2], Elisabeth Eger[1], Judith E.[1,2], Farnberger, Jakob Pletz[1], Anna Żądło[1]-Dobrowolska, Somayyeh Gandomkar[1], Adam Simon[3], Gideon Grogan[4], Kendall Houk[3]

FORMATION OF C-C BOND AND CHIRAL AMINES USING BIOCATALYSIS

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[3]University of California Los Angeles, USA
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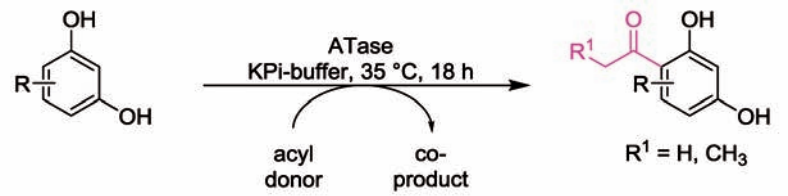
A biocatalytic Friedel-Crafts like reaction was recently enabled by the choice of suitable acetyl donors [1, 2]. To extend the scope of acyl groups to be transferred, the structure of the enzyme was solved and engineered. The engineered enzyme accepted beside the acetyl moiety now also sterically significantly more demanding groups.



Scheme. Biocatalytic Friedel-Crafts reaction using non-natural acyl donors

The Pictet-Spengler reaction of tryptamine and aldehyde enables a C-C bond formation leading to chiral amines, β-carbolines. The strictosidine synthase has been described to transform tryptamine and secologanin to the corresponding (*S*)-product [3, 4]. To our surprise we recently found out, that small aldehydes such as isovaleraldehyde are transformed to the corresponding (*R*)-product in essentially optically pure form. This enabled to short-cut otherwise long synthetic routes. Solving the crystal structure did not lead to a clear explanation why the enzyme gives the (*R*)- instead of the expected (*S*)-product; fortunately combining forces with MD-simulations led to an explanation.

Various biocatalytic methods leading to optically pure amines have been developed [5]. Transaminases have become an established method for the amination of ketones as a key step in the synthesis of active pharmaceutical ingredients. For the synthesis of the blockbuster pregablin, we recently engineered various transaminases to achieve high optical purity of the product [6].

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Felix Krujatz[1], Thomas Walther[1], Anja Lode[2], Ashwini Rahul Akkineni[2], Michael Gelinsky[2], Erik Trampe[3], Klaus Koren[4], Michael Kühl[3], Juliane Steingröwer[1]

GREEN BIOPRINTING - A TOOL FOR CREATING GREEN 3D-CELL/MATRIX

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3D-Bioprinting, additive manufacturing with integrated living cells, is a strong field of research mainly in tissue engineering and regenerative medicine. The technology of Green Bioprinting, developed by interdisciplinary researchers of TU Dresden, stands for a new approach combining the fields of additive manufacturing technologies, biotechnology, optical sensors and material & medical science. The structural embedding of cells within a hydrogel environment protect the production hosts from shear stress, improves the separation of cells from the medium and allows the development of different process strategies.

The talk will discuss the perspectives of creating structured 3D-immobilization matrices for microalgae and plant cells and their potential medical and biotechnological applications. A special focus will be on the selection of appropriate printing matrices for particular cell types and material properties, and on monitoring cellular health and growth in the scaffolds using optical technologies, e.g. fluorescence microscopy and optical active sensor-nanoparticles which can be applied to viszualize respiration and photosynthetic processes within the hydrogel environment.

Green Bioprinting offers a wide range of medical and biotechnological applications as well in basic research (e.g. research on symbiotic living organisms, e.g. quorum sensing, artificial construction of natural multi-specie environments, local- and time resolved analysis of cell properties in response to external stimuli) as applied research in medicine and biotechnology (multi-step metabolic bioreactions, combination of different materials and types of cells, e.g. microalgae as natural oxygen source for human cells).

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Vladimir Mrša, Amir Sk. Hossain, Antonija Grbavac, Mateja Lozančić, Renata Teparić

SURFACE DISPLAY OF PROTEINS IN YEAST – FROM UNDERSTANDING BASIC CONCEPTS OF CELL WALL BIOSYNTHESIS TO CELL SURFACE ENGINEERING

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Yeast cell wall is a complex extracellular organelle that requires sophisticated molecular mechanisms for its biosynthesis and remodeling. Most of the biochemical reactions involved in these cellular events have been revealed in the last several decades but their precise regulation is still largely unknown. It includes formation of regulatory protein complexes at the cell surface and proteolytic activation of, most probably, sets of proteins whose role is to a large extent still unexplained. Studies of microbial cell envelopes and particularly cell surface proteins and mechanisms of their localization brought about new biotechnological applications of gained knowledge in surface display of homologous and heterologous proteins. By fusing surface proteins, or their anchoring domains with different proteins of interest their so called genetic *immobilization* is achieved. Hybrid proteins are engineered in a way that they are expressed in the host cells, secreted to the cell surface and incorporated into the wall/envelope moiety. In this way laborious and often detrimental procedure of chemical immobilization of the protein to a solid matrix is avoided by letting the cells do the whole procedure. Both bacterial and yeast cells have been used for this purpose and a number of potential biotechnological applications of surface displayed proteins have been reported. Examples range from microbial whole cell biocatalysts, biosorbents, biosensors and biostimulants development to design and screening of protein and peptide libraries. When surface immobilized enzymes are used, substrates do not need to cross membrane barriers, i.e. enzymes are free to access any externally added substrate. Thus, often complex and expensive purification of enzymes used on an industrial scale is bypassed. In addition, the multi-step transformation can be performed using microbial cells displaying different enzymes that catalyze cascade reactions. In recent years particular attention has been paid to yeast systems for surface display of proteins since most yeasts are generally regarded as safe (GRAS) microorganisms, yeast cell walls are capable of binding more proteins, and the cells are bigger. Besides, yeasts are generally more suitable for expression of proteins originating from higher eukaryotes. In this talk our current knowledge on molecular mechanisms for yeast cell wall biosynthesis will be summarized. Besides, the application of knowledge gained through rather basic molecular research for surface display of proteins on yeast cell surfaces and their use in biotechnology will be discussed.

Muhammad Ismail[1], Aleksandra Zambrowicz[2], Łukasz Bobak[2], Marcin Sieńczyk[3], Balbina García-Reyes[4], Uwe Knippschild[4], Tadeusz Trziszka[2], Michał Zimecki[5], Timo Burster[6]

LACTOFERRIN AND YOLKIN-DERIVED PROTEINS INCREASE THE PROTEOLYTIC CAPACITY OF THE SERINE PROTEASE CATHEPSIN G IMPORTANT FOR AN IMMUNE RESPONSE

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Neutrophils secrete serine proteases, including cathepsin G (CatG), as a first cellular immune response against pathogens. CatG secreted at the site of inflammation has several functions, for instance, degrades pathogen-derived proteins, processes chemokines and cytokines, and plays an important role in antigen processing in the adaptive immune response.

We found that lactoferrin (LF) and yolkin, which is a polypeptide complex naturally occurring in hen's egg yolk, enhanced the proteolytic activity of CatG. The data provided show that both LF and yolkin change the substrate selectivity of CatG, while combination of LF and yolkin inhibits the proteolytic activity of CatG. In addition, CatG, LF, and yolkin effectively reduce the cell viability of glioblastoma cell line in a proteolytically independent manner. Furthermore, LF upregulates cell surface major histocompatibility complex class I (MHC I) molecules on immune- and glioblastoma cells important for an immune response. In conclusion, we describe novel biochemical properties of LF and yolkin.

SHORT COMMUNICATIONS

Session 1
MICROORGANISMS
Lecture 1.1

Zbigniew Lazar[1,2], Nian Liu[2], Peng Xu[2], Kangjian Qiao[2], Gregory Stephanopoulos[2]

WAX BIOSYNTHESIS BY *YARROWIA LIPOLYTICA*

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Yarrowia lipolytica are one of the most studied nonconventional yeast. They are capable of producing valuable metabolites, such as organic acids, polyhydroxyl alcohols, aromas and high levels of extracellular proteins. The most peculiar feature of this yeast is their ability to accumulate high concentrations of intracellular lipids which in wild-type strains reach up to 20% CDW (Cell Dry Weight) and in genetically-modified ones this value may reach up to 90% CDW. Processes with this microorganism were granted by FDA (Food and Drug Administration) a GRAS (Generally Recognized as Safe) status what allows them to be developed on industrial scale.

The ability of *Y. lipolytica* to accumulate high amounts of lipids, especially from hydrophobic substrates or by-products from other industries (e.g. glycerol) is a very promising starting point for biosynthesis of lipid derived compounds, such as waxes. Wax esters are neutral lipids, composed of long chain fatty acids and long chain fatty alcohols, which can serve as ingredients for personal care products, lubricants or coatings. We have cloned previously identified acylCoA reductase from *Marinobacter aquaeolei* VT8 (Maqu_2220) and fatty acylCoA synthetase from *Escherichia coli* (EcFadD), genes able to produce fatty alcohols in *Y. lipolytica*, in concent with wax ester synthase from *Arabidopsis thaliana* (WAX2), *Simmondsia chinensis* (WS1) and *Homo sapiens* (AWAT2). Only the human gene was able to produce active version of wax synthase in *Y. lipolytica.* During flask cultures the the *WAX*$^{+}$ transportants produced up to 2.4 g/L of waxes with a yield of 0.78 g/g CDW. Although this was the highest concentration of waxes produced by the transformants, full capacity of *Y. lipolytica* to synthesize waxes was not yet reached. Wax esters turned out to be toxic to the cells and inhibited their growth. Due to that, elimination of wax toxicity using different synthetic biology approaches was applied and is currently under investigation.

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PHYMET2 – DATABASE TOOL FOR GENETIC AND PHENOTYPE ANALYSIS OF MICROORGANISMS

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Increasing amount of information concerning growth conditions for microorganisms and genetic information requires new approach in data storage and analysis technology. Database technology may help in data organization and analysis, however design of friendly interface is required for majority of possible users. Moreover, finding correlations between sequence comparison and phenotype data is not an easy task. To solve this problems PhyMet[2] (Phylogeny and Metabolism of Methanogens) was created.

In present version PhyMet2 include phenotypic data describing 153 species of methanogenic archaea obtained from scientific literature and sequences from NCBI database. The gathered data include: morphology; temperature, alkalinity and salinity tolerance range; growth rate and nutritional requirements.

Relationships between phenotype and sequences are found with N-gram analyzer. This algorithm was successfully used for search for optimal culture conditions for uncultured methanogenic archaea based on analysis of 16S RNA sequence (in press EMEMR).

The PhyMet[2] was tested on relatively small group of microorganisms, however we would like to further develop this tool. The aim of the project is the application of PhyMet[2] in analysis of different aspects of microbial activity. To achieve it, the following improvements are required:

– Introduction of microorganisms other than methanogenic archaea,
– Analysis of sequences other than 16S RNA,
– Incorporation of traits other than growth requirements.

Our final goal is the construction of universal platform dedicated to storage and analysis of data describing different microorganisms. To expand the project, cooperation with scientific society is essential. To facilitate the future collaboration, currently developed version of PhyMet[2] has the user interface for introduction of new strains data.

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THE ROLE OF A NEWLY IDENTIFIED ISOMERASE FROM *YARROWIA LIPOLYTICA* IN ERYTHRITOL CATABOLISM

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Erythritol is a natural sweetener produced by microorganisms as an osmoprotectant. It belongs to the group of polyols and it can be utilized by the oleaginous yeast *Yarrowia lipolytica*. Despite the recent identification of the is transcription factor of erythritol utilization (*EUF1*), the metabolic pathway of erythritol catabolism remains unknown. In this study we identified a new gene, *YALI0F01628g*, involved in erythritol assimilation. *In silico* analysis showed that *YALI0F01628g* is a putative rpiB isomerase and it is localized in the same region as EUF1. qRT-PCR analysis of *Y. lipolytica* showed a significant increase in *YALI0F01628g* expression during growth on erythritol and after overexpression of *EUF1*. Moreover, the deletion strain ΔF01628 showed significantly impaired erythritol assimilation, whereas synthesis of erythritol remained unchanged. The results showed that *YALI0F1628g* is involved in erythritol assimilation; thus we named the gene *EYI1*. Moreover, we suggest the metabolic pathway of erythritol assimilation in yeast *Y. lipolytica*.

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BIOLOGICAL PREPARATION CONTAINING EXTRACELLULAR METABOLITES OF *DEBARYOMYCES HANSENII* YEAST DESIGNED TO PROTECT APPLE FRUITS AND LEAFS FROM FUNGAL DISEASES

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Venturia inaequalis is a fungus causing the disease of apple trees called apple scab. Its development on leaves significantly weakens the plant, while on fruits causes black spots disqualifying them in the eyes of consumers. Moreover, the growth of other fungus – *Monilinia fructigena* on apples in the form of brown rot leads to significant crop losses during the storage. Therefore, strict chemical protection of apple trees against these pathogenes is used in all industrial orchards. However in recent years, the awareness of danger resulting from the widespread use of chemicals in food production chain has grown significantly. In response EU regulations were created ordering the adoption of integrated plant protection methods in industrial horticulture (EU Directive 2009/128 / EC and Regulation No. 1107/2009). These regulations recommend agrotechnical treatments and biological origin preparations before chemicals.

We have developed a new antifungal biological preparation for apple trees containing extracellular metabolites including killer toxins secreted by Debaryomyces hansenii, the yeast commonly found in cheese. The presented technology consists of: yeast cultivation in a medium based on by-products of the food industry (beetroot molasses and corn steep liquor), separation of biomass and concentration of cell-free culture liquid by nanofiltration. In order to evaluate the suitability of the developed preparation in the orchard, an industrial scale bioreactor culture was made at Skotan S.A. in Czechowice-Dziedzice, Poland. The concentrated liquid was sprayed on apple trees of Alwa variety in the orchard belonging to WUELS. The effectiveness of the formula was evaluated on the degree of plant organs infection and growth parameters of apple trees during the season.

The yeast preparation showed high effectiveness on apple trees against apple scab, comparable to chemical protection (89.7–96% of healthy leaves). Analysis of crop size and quality also showed the suitability of the toxin-based formula; about 2.5-time increase in fructification was recorded compared to non-protected trees. The use of preparations of D. hansenii origin significantly extended the storage time of fruits; brown rot losses after 5 months were comparable to chemical protection, while fruits from control trees were completely destroyed after only 30 days of storage. Interestingly, a significant increase in the average size of fruit due to biological protection was also observed.

The production technology is protected by the patent of the Patent Office of the Republic of Poland (decision DP.P.412635.18.bmia dated 7/05/2018) and is registered for protection in the European patent office.

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DESIGN AND CHARACTERIZATION OF NEW LIPID NANOPARTICLES BASED ON PHOSPHOLIPID CONJUGATES OF BIOLOGICALLY ACTIVE ISOMERS OF CLA AND POLYPHENOLIC ACID DERIVATIVES

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Nowadays, the use of natural compounds with confirmed biological activity in designing new drug formulations or dietary supplements is becoming more and more popular. Natural compounds due to their origin are considered as GRAS and gain greater trust of consumers and patients. Although many natural compounds, exhibit high biological potential in *in vitro* cytotoxic studies their activity usually decreases when used in human trials which is explained by their limited bioavailability and very complex metabolism of human body. Therefore ways to overcome these limitations has been developed, for example by binding biologically active compound (BAC) to a lipophilic molecule [1].

Covalently bonded BAC with phospholipid (PL-BAC) such as phosphatidylcholine may have several advantages including good oral bioavailability in the organism, improved targeting to the lymphatic system and enhanced activity. Moreover their functionality can be additionally increased by using them in the designing of lipid nanocarriers such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). Binding hydrophilic BAC with phospholipid increases its lipophilicity and enhance its affinity to lipid core of the carriers and thereby reduce BAC leakage from the system. Wisely designed PL-BAC is not only a lipophilized active element of lipid core, but also could act as a functionalized surfactant.

In this communication we would like to present our recent research and future perspectives on design and characterization of lipid nanocarriers (SLN and NLC) fabricated with biological active phosphatidylcholine conjugates of either *c*9,*t*11 conjugated linoleic acid (CLA), *t*10,*c*12 CLA or phenolic derivatives such as p-anisic and veratric acids which were synthesized by our research group [2] Factorial design method was used to optimize size, polydispersity index (PdI) and zeta potential (ζ) of SLN and NLC. Developed nanocarriers were characterized in terms of shape, stability and biological activity. The *in vitro* cytotoxic tests were performed on human cancer epidermoid carcinoma (A431 and MeWo) using trypan blue staining and MTT assay.

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Michal Godula

SEARCHING THE NEEDLE IN A HAYSTACK – USING THE Orbitrap™ HIGH RESOLUTION MASS SPECTROMETRY AND NOVEL DATA MINING TOOLS IN UNKNOWNS SCREENING, IDENTIFICATION AND CONFIRMATION

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Screening of toxins, contaminants and residues in food is of great importance in regulated environments such as food control labs, contract labs and routine quality control. Due to the broad variability of physico-chemical properties of pesticides the trend is to employ simple sample preparation procedure to maintain the recovery of the broad range of analytes and to streamline the sample preparation procedures to lower analysis costs and increase lab throughput. This unavoidably leads to the fact that final extracts injected into the chromatographic systems contain significant amounts of coextracts. For the chromatographic determination it is therefore essential to apply the systems with high selectivity and low achieved detection limits.

Traditionally the analysis of low levels of pesticides has been carried out using selected reaction monitoring (SRM) scanning using triple quadrupole mass spectrometer instruments. This approach has significant advantages with respect to achieved performance but also certain limitations such as limited number of compounds per analysis, little possibility to scan for unknown compounds at high levels and necessary system optimization to run specific set of compounds.

Because of these limitations, in residue analysis there is currently a trend towards applying the full scan MS acquisition experiments using instruments delivering high mass accuracy and resolution. High resolving power of the mass spectrometers based on Orbitrap™ and their ultimate mass accuracy provide unique advantages in the screening and quantitation of low levels of contaminants in complex food matrices.

The presentation will demonstrate how the recent developments in the instrumental techniques allow to improve the methods used in food labs to detect low levels of various residues in foods. The application of techniques of high resolution data acquisition and advanced data mining, using state-of-the-art software tools such as mzCloud™ and Compound Discoverer™, will be demonstrated by examples and explanations.

Session 2
BIOSYNTHESIS, BIODEGRADATIONS & BIOTRANFORMATIONS
Lecture 2.1

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FUNCTIONAL, STRUCTURAL AND TOXICOLOGICAL CHARACTERISATION OF TWO BIOPOLYMERS PRODUCED BY MARINE BACTERIA

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The ability of marine bacteria to produce biosurfactants or biopolymers (such as exopolysaccharides – EPS) has been extensively studied over the past four decades (Gutiérrez et al. 2007, Schulz et al. 1991, Tanaka et al. 2008). These findings were beneficial especially for bioremediation where biosurfactants were shown to enhance the biodegradation of crude oil and its derivatives by increasing the uptake of the hydrocarbons by the bacterial cells (Brooijmans et al. 2009, Christofi and Ivshina 2002).

We present work from the MARISURF EU Horizon 2020 project (www.marisurf.eu), where a collection of over 500 marine bacterial strains, isolated from a number of marine reservoirs, has been screened for their ability to produce novel surface active agents (SAs). Four main properties, emulsification, gelling, foaming and/or surface tension reduction ability were selected as the criteria for identifying the most promising microorganisms and their products.

The presented biopolymers under their working names Biopolymer A and Biopolymer B were extracted from the two corresponding non-pathogenic marine strains – Strain A and Strain B. Both biopolymers were characterized by very high emulsifying and foaming activity and stability. Additionally, their gelling activity was detected and confirmed through the rheological tests. The toxicological analyses suggest that isolates from Strain A and Strain B are not associated with any significant levels of cell death in either of the *in vitro h*uman skin and human liver models up to the tested 1mg/ml concentration. The 1H-1D NMR analyses suggest that Biopolymer A is most likely a lipopeptide, while Biopolymer B a glycoprotein or proteoglycan.

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INFLUENCE OF NEGATIVE PRESSURE ON ACTIVITY OF ACTIVATED SLUDGE BACTERIA

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Reduction of pressure for a short time can be used for degassing of activated sludge while wastewater treatment. Degassing is performed before its discharge to secondary clarifier and improves the settling properties of activated sludge. During the degasification the activated sludge flocs are believed to be destroyed due to gas bubbles escaping the mixture and reflocculated suddenly after the pressure above the mixed liquor is atmospheric. The physical process is known to enhance the nutrients removal efficiency. However, the effect of negative pressure on bacteria is not recognized and improvement of nutrients removal is explained just by the increase of bacteria involved in the treatment process as a consequence of increased suspended solids concentration in the reaction chamber (resulting from better settling properties).

The aim of the research was to determine the effect of negative pressure on activity of activated sludge by means of different activity tests.

Among the tested overall and specific activities were nitrification, denitrification and dephosphatation activity. Both the duration and value of negative pressure were tested.

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PRODUCTION OF LIPIDS BY *YARROWIA LIPOLYTICA* FROM WASTE MATERIALS

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Nowadays, when fossil fuels are likely to soon be exhausted and plant energy resources will compete with food production for farmland, microbial lipids might become one of potential feedstock for production of fuels and chemicals. To make a biotechnological production more economically available and widely used new technologies must be developed to reduce costs on energy consumption, freshwater and substrate usages. Thus, important is to use for biotechnological production of microbial lipids the low-cost carbon sources like crude glycerol or lignocellulose materials. Glycerol is produced by several industries like fat saponification or biodiesel production, whereas lignocellulosic materials are mainly produced by agriculture. Additionally, bio-processes that require the growth of microorganisms in large volumes require enormous amounts of water. In bio-industry mostly freshwater is used, which cause the competition with the constantly decreasing access to clean and good quality freshwater. For this reason, the possibility of intracellular lipids production by oleaginous yeast *Yarrowia lipolytica* in seawater-based medium was investigated. The lipid production by yeast was investigated in seawater-based media containing different carbon sources including waste materials. Crucial to the economic worthwhile of these process is production high lipid titers by properly prepared strains. Lipid synthesis in cells depends on activity of various enzymes. In this study, we aimed to examined the impact of overexpression of the genes involved in fatty-acid synthesis and metabolism of various carbon feedstocks. To enhance production of lipids from glycerol, we overexpressed the GUT1 gene coding glycerol kinase, first step in glycerol utilization. Next, to direct carbon flow into lipid production we overexpressed the SCT1 gene encoding G3P-acyltransferase. Subsequently, to improve the SCO production, we additionally overexpressed DGA1 gene encoding DAG-acyltransferase involved in the last step of triglycerides synthesis.

This work demonstrates that effective metabolic engineering may create biological platform for efficient lipid production by yeast from inexpensive renewable resources and in seawater-based medium for production of fuels and chemicals.

Session 3
PLANT & ALGAL BIOTECHNOLOGY
Lecture 3.1

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INNOVATIVE LIGHT SYSTEM FOR IMPROVED PLANT PRODUCTION: IMPACT ON ROS/HORMONAL HOMEOSTASIS

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Plants are constantly exposed to various environmental factors, which affect their growth, development and productivity. Light and water are accessibility are the most important, therefore diurnal and seasonal changes in light quantity and quality and water uptake regulate such important crop traits as yield, water use efficiency and biomass production. Here, we present the new prototype of LED lamps (ISOR) accordingly to the patented technology (Karpiński and Szechyńska-Hebda, USA Pat. 9131645). In these light-regulated processes, multiple hormonal pathways are often modulated by light to mediate the developmental changes. Although interactions between light and hormone signaling pathways have long been observed, recent studies have advanced our understanding by identifying signaling integrators that connect the pathways. Light signaling components and they link to the signaling of phytohormones, such as gibberellin (GA), abscisic acid (ABA), auxin and cytokinin, in regulating photomorphogenesis and seed germination. This work focuses on the positive impact of ISOR system on plant growth, development and seed production through the regulation of ROS/hormonal homeostasis.

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LSD1, *EDS1* AND *PAD4* REGULATE BIOMASS PRODUCTION IN HYBRID ASPEN (*POPULUS TREMULA X TREMULOIDES* MICHX.)

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Orthologues of *Arabidopsis thaliana LESION SIMULATING DISEASE 1 (LSD1), ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1)* and *PHYTOALEXIN DEFICIENT 4 (PAD4)* genes were silenced in wild type hybrid aspen clone T89 using RNAi technology. Exploiting Multisite Gateway technique, transgenic plants with reduced expression of *LSD1, EDS1, PAD4* genes and in combination (*LSD1*/*PAD4* and *EDS1*/*PAD4)* were generated. Silencing of *LSD1*, *EDS1* and *PAD4* genes in the range from 0,22 to 0,79 resulted in modified growth and biomass production of trees under both, *in vitro* and greenhouse conditions. Single gene silencing resulted in higher biomass accumulation and more efficient growth and development, than in non-transgenic plants cultivated under identical conditions. Under *in vitro* conditions, one month old *LSD1/PAD4–RNAi* trees had greater biomass production, when compared to *EDS1/PAD4–RNAi* and wild type trees. Morphological analysis of *EDS1/PAD4–RNAi* lines showed reduced length of the main stem and improved formation of lateral branches. In the same growth conditions number of lateral branches of *LSD1/PAD4-RNAi* lines and wild type trees were comparable. Surprisingly, under greenhouse conditions total biomass accumulation was the greatest in *EDS1/PAD4–RNAi* lines, mainly as a result of the highest thickness of main stem. More efficient growth of *EDS1/PAD4–RNAi* lines was accompanied by greater water use efficiency. Transgenic trees with improved water use efficiency and biomass production can open opportunities for successful cultivation of plants under stress and semi-stress conditions *e.g.* can be recommended in areas with poor irrigation. In order to confirm this statement, we performed chlorophyll *a* fluorescence analysis. *EDS1/PAD4–RNAi* lines had a lower non-photochemical quenching (NPQ) value compared to *LSD1/PAD4–RNAi* lines and wild type plants. It suggests that *EDS1/PAD4–RNAi* trees exhibit the ability to perform more efficient photosynthesis and can grow in more successful manner than the control plants that are not genetically modified. *EDS1* could be a key regulator of these features. In conclusion, our results demonstrate that *EDS1*, *PAD4* and *LSD1* are involved in the regulation of photosynthesis and plant biomass production.

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BIOTECHNOLOGICAL IMPROVEMENT OF FLAX PLANTS-TOWARDS INCREASED PATHOGEN RESISTANCE COUPLED WITH IMPROVED PROPERTIES OF FLAX PRODUCTS

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Commonly cultivated in our climate zone, flax (*Linum usitatissimum* L.) is an annual plant originating from the Middle East, considered as one of the oldest cultivated plants, which were the source of fiber and oil. It has a wide range of applications, starting from the use of fiber to produce ropes and textiles, through the use of oil as a diet supplement to oil's application in
the cosmetic and chemical industry. Also the compounds considered as waste-products (shives, seedcake) are the source of valuable components like phenolics, which make flax perfectly suitable to the "zero-waste" slogan, because all its parts may be used in various branches of industry. A necessary condition for the renewal of mass cultivation of flax, is a constant concern for seed and fiber quality, which can be achieved by strengthening the resistance of flax, because the plant is susceptible to pathogenic infections. The greatest losses in flax cultivation are caused by the fungal infections. Vascular wilt caused by *Fusarium oxysporum* can account for up to 20% of the annual losses in flax cultivation. Resistant flax cultivars have been used to manage the disease, but the resistance varies, depending on the interactions between specific cultivars and isolates of the pathogen. The research into plants resistance to pathogens has been going for many years and over the time many ways to improve plant resistance to pathogens were established. Although the increased resistance to pathogen could be achieved via various methods we are particularly interested in those which, apart from the improvement in resistance, improve the properties of flax products. This may facilitate their wider application including in medicine and cosmetics. Because of that we concentrated our research on secondary metabolite pathways active in flax. Using differential library screening several genes from various pathways possibly involved in flax resistance to *Fusarium* were identified. Those included those from phenylpropanoid, terpenoids, polyamines and pectin metabolism pathways. Subsequently we studied the transcriptomic and metabolomics response of those pathways in more detail selecting some genes for further analysis. Several varieties of transgenic plants were created to confirm the importance of selected genes in pathogen resistance and to evaluate the biomedical value of obtained products.

Even though our understanding of mechanism and pathways involved in response to pathogens is constantly growing, there still remain some questions which need answering. To further expand our understanding of the flax response to pathogens we recently performed full transcriptome analysis flax seedlings after *Fusarium* treatment using RNA-seq method in early stages of infection. This gave us the more in depth understanding how the plants respond to attempted infection, which can help plan future breeding programs to improve resistance.

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PERSPECTIVES OF FLAX IMPROVEMENT BY BIOTECHNOLOGICAL METHODS

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Flax (*Linum usitatissimum* L.) is the crop plant, cultivated in temperate climate for fibre and oil. The cultivated species of flax are characterized by low genetic variability, resulting also from the fact that flax is self-pollinated plant. Traditional methods for breeding and crossing are long-term and ineffective. Thus application of methods for genetic engineering of flax is desirable and important way to increase its genetic variability and improve the qualitative and quantitative properties of plants. Transgenesis, suspension cultures (derived from genetically modified flax) and flax hairy root cultures have been investigated and discussed in the aspect of application for flax improvement. It should be pointed out that establishing the stable cultures remains promising way to develop new properties in plants and produce significant metabolites in future. Novel strategies for application of genetically modified flax plants will be presented.

Session 4
ENZYMES & PEPTIDES
Lecture 4.1

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BIOPEP-UWM DATABASE OF BIOACTIVE PEPTIDES – STATUS IN 2018

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The BIOPEP-UWM database (provider: University of Warmia and Mazury in Olsztyn, Poland) is widely used as a source of information concerning biological activity and taste of peptides from food proteins. Over 200 articles, describing in silico studies involving the database or experiments supported by the above bioinformatics tool, was published to date. BIOPEP-UWM database is used for construction of profiles of potential biological activity of protein fragments (mainly of food origin), simulations of proteolysis and searching for information about bioactivity of components of protein hydrolyzates, identified using HPLC and mass spectrometry.

The aim of presented work was to provide enhanced opportunities for quantitative characteristics of proteins as precursors of bioactive and sensory peptides, parameters characterizing possibility of release of such fragments during proteolysis as well additional search options.

New options include batch processing, enabling work with up to 30 protein or peptide sequences, submitted in FASTA format. Batch processing possesses the following functions: building of profiles of potential biological or sensory activity of proteins, simulation of proteolysis by selected enzymes, finding active fragments among predicted products of proteolysis and calculation of quantitative parameters characterizing proteolysis. Information about specificity of proteolytic enzymes was updated to take into account recent information annotated in the MEROPS and CutDB databases.

The new search possibilities include "exact match" option for sequence-based search, enabling to find single peptide, identical with query and search based on InChIKey. The last one is unique code designed for annotation of chemical compounds. INChIKey contains always 27 characters and may be used as a query for searching via Google™ or via specialized programs such as Chemical Translation Service. Introduction of InChIKeys and other chemical codes (SMILES, InChI) is aimed on improvement of BIOPEP-UWM compatibility with cheminformatics tools [1, 2].

The BIOPEP-UWM database contains recently (2018.03.19) data about 3643 bioactive peptides and 478 sensory peptides or amino acids sequences. Database enables simulation of proteolysis on the basis of up to three of 33 proteolytic enzymes.

The database is available at the following website: http://www.uwm.edu.pl/biochemia/index.php/pl/biopep

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TESADH W110A I86A C295A AS THE RACEMISATION CATALYST FOR A BIS ENZYMATIC DYNAMIC KINETIC RESOLUTION

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For a successful dynamic kinetic resolution (DKR) of secondary alcohols employing enzymes only, the most challenging part is the racemisation catalyst. Due to the fact that biochemical processes are predominantly stereospecific, alcohol racemases represent a very small group of enzymes (e.g., mandelate racemase, lactate racemase, acetoin racemase). Application of those enzymes in DKR is limited by a narrow substrate scope of α-hydroxy carbonyl derivatives [1]. In order to emulate a racemase activity, in analogy to a transition-metal catalysed racemisation, a non-stereoselective dehydrogenase is required to interconvert a chiral centre to a prochiral intermediate. However, a vast majority of papers describing enzymes relate to their high enantioselectivity or enantiospecificity, therefore there are only few reports of non-stereoselective enzymes [2, 3].

Thermoanaerobacter ethanolicus secondary alcohol dehydrogenase (TeSADH) is a thermostable and enantioselective enzyme that is not active towards any aromatic or more sterically demanding substrates [4]. Introduction of specific mutations inside the catalytic pocket resulted in variants with broadened substrate scope e.g., C295A with activity towards branched aliphatic substrates, W110A or I86A with activity towards aromatic substrates. Surprisingly, W110A and I86A variants share a different stereoselectivity [5–7].

Herein, we would like to present results of our investigation of the TeSADH W110A/I86A/C295A triple variant as the racemisation catalyst and its possible application in a dual enzymatic dynamic kinetic resolution.

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VALORIZATION OF CHICKEN FEATHER WASTE WITH NEW ISOLATES OF KERATINOLYTIC *COCCI*

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The degradative potential of keratinolytic bacteria is considered as an effective means for valorization of feather waste or other keratinous by-products through biotechnological processes. New feather-degrading bacteria were acquired after isolation from living poultry. One isolate, identified as *Kocuria rhizophila* was selected for the evaluation of feather bioconversion process at flask-culture scale, and maintained under accession number PCM 2931. Throughout cultures in feather-containing medium the strain produced moderate proteolytic activity, however, significant concentration of free amino groups and soluble proteins, accompanied by high content of reduced thiols and fast liquefying of the substrate, confirmed its effectiveness. The process was further enhanced through a two-step procedure, comprising a Plackett-Burmann screening design, followed by a Box-Behnken optimization desing. Optimum concentration of most influential medium components (feathers, MgSO4 and KH2PO4) was defined to produce the maximum output of 0.66 mg/mL soluble proteins. The raw feather hydrolysate was rich especially in glutamic acid, proline, cysteine and aspartic acid, while the supernatant contained mainly phenylalanine, arginine, histidine, aspartic acid. Additionally, the hydrolysate exhibited anti-oxidative properties, as determined with ABTS, DPPH and FRAP. Subsequent treatments of the raw broth resulted in the further increment in anti-oxidative activities. The properties of the new *K. rhizophila* isolate allow for its potential use in efficient production of feather hydrolysates for various prospective applications.

POSTERS

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COMPARISON OF LIQUID AND SEMI-SOLID STATE FERMENTATION FOR GROWTH AND SPRAY-DRYING OF PROBIOTICS

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Spray-drying is one of the techniques applied in preservation of probiotic bacteria. One of the most commonly used solution for preservation of probiotic bacteria is separation of cells grown in liquid medium, its washing with phosphate-buffered saline (PBS), and then spray-drying of biomass suspended in protective medium. In this study, two methods of probiotic bacteria batch cultivation were compared with regard to final count of bacteria in post-culture medium, viability rate, and count of viable cells during process of preservation. The first cultivation method was based on growth of bacterial biomass in MRS liquid medium. Following stages of biomass preparation proceed as mentioned above. In the case of semi-solid fermentation procedure, bacteria cells were cultivated in corn flour-based medium. The main advantages of this technique include: (1) its simplicity of operation, (2) low-cost components, and (3) high cell count in post-culture medium. Since the biomass was attached to the corn flour granules, spray-drying process was performed without *biomass-separation step.*

Bioreactor cultivations of *Lactobacillus plantarum* LOCK 0860 were conducted in a bioreactor vessel (Sartorius Stedim, Germany), in a working culture volume of 4 L. Temperature, mixing, and pH value were stably maintained throughout the process at 30°C, 150 (liquid medium) or 500 rpm (semi-solid medium) and 6.4, respectively. The pH value was regulated through automatic addition of an neutralizing agent (20% NaOH). The cultivations were continued for 8 (liquid medium) or 20 h (semi-solid medium). Washed biomass from liquid fermentation procedure, and post-cultivation semi-solid medium were spray-dried in the same process conditions(T_{inlet} = 180°C; T_{outlet} = 80°C). In addition, the optimization of protective medium for spray-drying of *L. plantarum* LOCK 0860 has been performed based on the statistical experiment design. Following substances were tested as the protective agents: monosodium glutamate, trehalose and skim milk powder.

The cell viability after the preservation process was apparently depending on the protective medium used. It was found that there is no protective medium that is optimal for both washed biomass from liquid fermentation procedure, and post-cultivation semi-solid medium. Both method of cultivation ensure obtaining high cell count, however during spray-drying there are differences in the reduction of viable cells. Higher $\log_{10}$ reduction (CFU ml^{-1}) was found in the case of semi-solid medium. Factors that could have an impact on the high level of biomass reduction during preservation process have been presented.

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ANTIPROLIFERATIVE ACTIVITY OF HOP FLAVONOIDS ON HUMAN CANCER CELL LINES *IN VITRO*

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The hop plant (*Humulus lupulus* L.) contains a number of flavonoids with potential anticancer activity, however little is known so far about the structural requirements that determine such an activity. We obtained five flavonoids: xanthohumol, dihydroxanthohumol, isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin, which along with naringenin and cisplatin were tested *in vitro* for the antiproliferative activity against eight cancer cell lines. Antiproliferative activity of these compounds against eight human cancer cell lines (breast cancer: MCF-7, T-47D, MDA-MB-231, ovarian cancer: A-2780, A-2780cis, prostate cancer: PC-3, Du-145, colon cancer: HT-29), as well as towards two normal cell lines (human lung microvascular endothelial and human mammary epithelial cell line) was investigated.

Xanthohumol and dihydroxanthohumol demonstrated higher antiproliferative activity than cisplatin against two breast cancer cell lines: T-47D and MDA-MB-231. 6-Prenylnaringenin was also more active than cisplatin towards T-47D cell line and the SI_B value was 6.87.

It was proved that tested flavonoids have antiproliferative activity against human cancer cell lines and that they are very selective.

The anticancer activity is related to the structures of flavonoids. Comparing the antiproliferative activity and cancer selectivity of tested compounds we came to the conclusion that there are the chalcones that are promising potential chemotherapeutics, especially to these cancer lines, to which they showed higher antiproliferative activity than

cisplatin (IC_{50} [μM]) and their selectivity indexes (SI) were much greater than one, i.e. T-47D, MDA-MB-231, and A-2780 cis.

The most promising compound concerning high antiproliferative activity against tested human cancer cell lines is xanthohumol, which is inexpensively accessible from waste (spent hops). However, in comparison to activity on normal cell lines (selective action) it seems that dihydroxanthohumol is even more interesting in context of using it as a potent and selective anticancer drug. As far as the antiproliferative activity is concerned, the presence of a prenyl group is necessary, whereas O-methylation of the hydroxyl group at C-5 increased the cytotoxicity of the compounds against all tested cancer cell lines. The prenyl group at C-8 increased the antiproliferative activity, with the exception of the breast cancer cell lines. For these lines, especially T-47D, it was 6-prenylnaringenin that proved to be more active (IC_{50} lower than for cisplatin) and more selective.

The results of our research provide valuable information, which after complementary *in vivo* study may be useful for designing new medicines that are safe and devoid of harmful side effects.

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CARBONIC ANHYDRASES (CAS) REGULATE BIOMASS PRODUCTION AND PHOTOSYNTHESIS IN HYBRID ASPEN (*POPULUS TREMULA X TREMULOIDES* MICHX.)

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Carbonic anhydrases (CAs) catalyse reversible interconversion of CO_2 and water into bicarbonate (BC) and protons and regulate concentration of CO_2 around photosynthetic enzymes. For wild type plants was performed an experiment to check bicarbonate influence on photosynthesis and carbonic anhydrase activity. Under high light conditions (350 μmol PAR m^2 s^{-1}, photoperiod 8/16h, 22°C) wild type plants were fertilized with 80 ml of 3 mM, 10 mM, and 20 mM $NaHCO_3$ (pH = 7). Control plants were treated with water at the same volume. Bicarbonate treatment resulted in lower maximum quantum yield of PSII (Fv/Fm) and quantum yield of PSII (ΦPSII) values and higher non-photochemical quenching (NPQ) value. Additionally, wild type plants fertilized with 3 mM BC had the greatest biomass production and produced more leaves. BC treatment in moderate concentration (3mM) caused an increase CAs activity, which is required in CO_2 homeostasis maintenance. Our results suggest that changes in chlorophyll *a* fluorescence parameters are not a stress indicator but can correlate with plant growth and development. We also tested the effect of BC fertilization on photosynthesis and CAs activity in double-silenced lines, which were obtained using RNAi technology. *LESION SIMULATING DISEASE 1* (*LSD1*), *ENHANCED DISEASE SUSCEPTIBILITY 1* (*EDS1*) and *PHYTOALEXIN DEFICIENT 4* (*PAD4*) genes were silenced in wild type hybrid aspen. Exploiting Multisite Gateway technique, transgenic plants with reduced expression of *LSD1*, *EDS1*, *PAD4* genes and in combination (*LSD1*/*PAD4* and *EDS1*/*PAD4*) were generated. BC treatment resulted in lower maximum efficiency of PSII (Fv/Fm) value in wild type plants but increased in *LSD1/PAD4–RNAi* lines. Wild type plants and *LSD1/PAD4–RNAi* lines had a significantly lower NPQ value compared to control (plants fertilized with water). *EDS1/PAD4–RNAi* trees exhibited greater stability of photosynthetic parameters. In order to confirm our results, we performed CAs activity measurement. BC treatment caused an increase CAs activity in every genotype but the highest CAs activity was observed in *EDS1/PAD4–RNAi* lines. Our results demonstrate that carbonic anhydrases regulate photosynthesis and plant biomass production.

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ANTIOXIDANT PEPTIDES RELEASED FROM β-CASEIN WITH THE USE OF NONCOMMERCIAL PROTEASES PREPARATIONS

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Milk proteins are a very rich source of biologically active peptides, which are released *in vivo* by proteases of digestive tract, as well as *in vitro* during hydrolytic processes occurring in fermented products or performed with the use of different proteolytic enzymes. β-casein fraction is known as a precursor protein of many peptides expressing different biological activities. As the amount and the activity of biopeptides released from certain protein substrate significantly depends on the protease used in the hydrolysis process, the aim of the study was to analyze the effect of three different, noncommercial proteolytic preparations on degradation of β-casein and on the release of antioxidant biopeptides.

The hydrolysis process of the substrate protein was conducted with the use of plant serine protease isolated from Asian pumpkin (*Cucurbita ficifolia*) and two yeast extracellular proteases (aspartic and serine) obtained from the liquid culture of *Yarrowia lipolytica*. Reactions were performed for 5h with serine proteases at the pH 7,5 in 0,1M Tris-HCl buffer and with the aspartic protease at pH 3,5 in glycine-HCl buffer. Protein degradation was controlled by the determination of the hydrolysis degree (DH) and the free amine group content. Obtained hydrolysates were fractionated by the RP-HPLC method. Their antioxidant activities, expressed as the ability to scavenge DPPH free radicals, ferric reducing power and chelation of iron ions Fe (II), were also determined.

Application of noncommercial proteases in β-casein hydrolysis confirmed that those enzymes caused deep degradation of the protein, what was observed by the high DH, which however was dependent on the type of the protease, it's dose in the reaction mixture and time of the hydrolysis. Both serine proteases express high activity against β-casein, the achieved DH level reached 35–40% after 5h of the process, whereas the aspartic protease was two times less active. In correlation to determined DH in obtained hydrolysates were also the free amino groups content and the RP HPLC peptide profiles.

The determined antioxidant activity increased with the time of the degradation process. The highest free radicals scavenging activity and ability to chelate iron ions were observed in hydrolysates obtained with plant protease, whereas the highest the level of ferric reducing antioxidant power was determined in hydrolysates produced with *Yarrowia lipolytica* serine protease. The use of aspartic protease for β-casein degradation resulted in the release of peptides with similar scavenging activity, but significantly lower than other activities.

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TWO-STAGE MICROBIAL TREATMENT OF BREWER'S SPENT GRAIN FOR ENHANCED EXTRACTION OF PROTEINS

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Brewer's spent grain (BSG) is a high-volume, low cost by-product from the brewing industry. It is composed mainly of the husk-pericarp-seed coat layers that covered the original grain, residue of starchy endosperm and walls of empty aleurone cells. The BSG contains hemicelluloses (28%), cellulose (17%), lignin (28%), lipids(10%) and nearly 20% of protein. Highly important is the presence of essential amino acids, raising the BSG nutritional value. Nowadays, BSG is mainly used as convenient animal fodder. However, high concentration of cell wall carbohydrates in BSG and its complex structure significantly hinder the availability of grain proteins for biotechnological applications.

To create new possibilities for the BSG exploitation, a two-stage microbial treatment was proposed. The solubilization of BSG by the combined action of various bacteria of arctic origin, followed by proteolytic action of *Bacillus cereus* PCM 2849, was studied. Bacteria used in a first phase were characterized by high cellulolytic, xylanolytic or polygalacturonase activity. Initially, grain cell wall carbohydrates were hydrolyzed in order to loosen grain cover layer. The level of released reducing sugars were estimated using dinitrosalicylic acid (DNS) method. In the second stage of treatment, BSG proteolysis was carried out and the level of free amino acids and soluble proteins were investigated.

Research showed that the two-stage microbial treatment strongly increased the concentration of released amino acids and proteins, comparing to the samples hydrolyzed solely by *B. cereus* PCM 2849. Obtained results give hope for novel routes of efficient BSG management and prove that further research is rational.

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ISARIA SPECIES EFFECTIVELY BIOTRANSFORM FLAVONOIDS INTO CORRESPONDING 7-GLYCOSIDES

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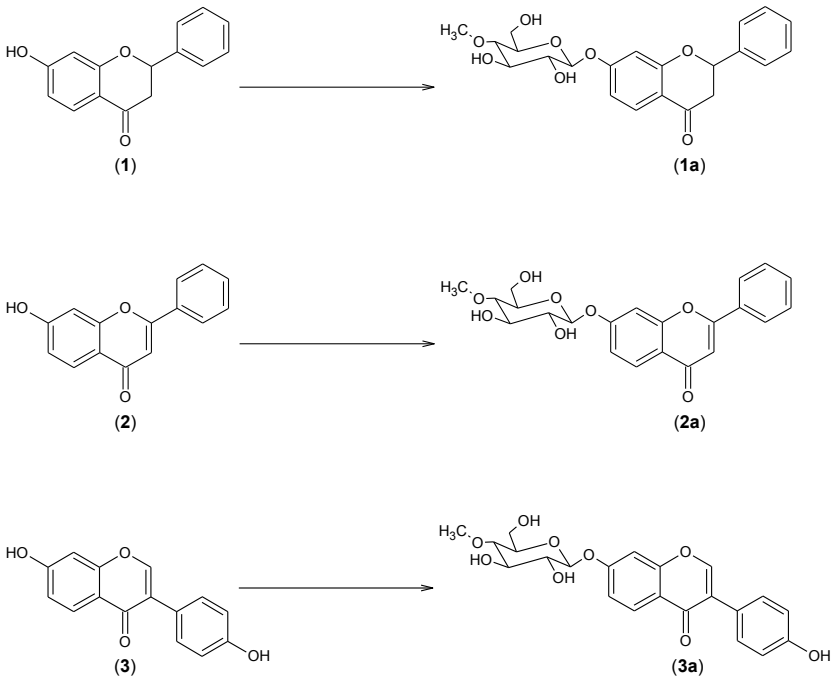
Biotransformations are a well-known tool to improve properties of compounds that can be used as pharmaceuticals and food additives. In the present study we used three flavonoid substrates with hydroxyl group at C-7 position (7-hydroxyflavanone (**1**), 7-hydroxyflavone (**2**) and daidzein – 4',7-dihydroxyisoflavone (**3**)) and strains of entomopathogenic filamentous fungi of the genus *Isaria*.



Scheme 1. Microbial glycosylation of 7-hydroxyflavanone (**1**), 7-hydroxyflavone (**2**) and daidzein (**3**) in *Isaria* sp. cultures

As a result, we obtained three flavonoid glycosides: flavanone 7-*O*-β-D-(4"-*O*-methyl)-glucopyranoside (**1a**), flavone 7-*O*-β-D-(4"-*O*-methyl)-glucopyranoside (**2a**) and 4'-hydro-xyisoflavone 7-*O*-β-D-(4"-*O*-methyl)-glucopyranoside (4"-*O*-methyldaidzin) (**3a**) with yields of 36, 16, and 15%, respectively in the culture of *I. fumosorosea* KCH J2.

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BIOTRANSFORMATION OF HALOLACTONES WITH TRIMETHYLCYCLOHEXENE RING

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Ionones are cyclic unsaturated ketones formed during degradation of carotenoids [1]. They are found in essential oils of various plants, such as rose oil [2]. They are also present in vegetables and fruits, such as berries or tea [3] and tobacco [4] leaves.

Structural analogues of ionones are generally odourless but they exhibit a number of other interesting properties. Numerous ionone derivatives isolated from different plants have a significant effect on the surrounding environment by demonstrating allelopathic activity [5, 6]. b-Ionone and its synthetic analogs and derivatives show anti-proliferative, anti-metastatic and pro-apoptotic activity [7].

Three halolactones were obtained from a commercially availableβ-ionone during a four-step chemical synthesis. These compounds were tested for their capability of converting into hydroxylactone by several fungal strains (*Fusarium* sp., *Absidia* sp., *Aspergillus* sp.). Four species of *Fusarium* transformed bromolactone into hydroxylactone by hydrolytic dehalogenation. It was found that bromo- and iodolactone were characterized by interesting odour activity.

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ENANTIOMERIC β-(2',5'-DIMETHYLPHENYL)-γ-BROMO-δ-LACTONES: CHEMOENZYMATIC SYNTHESIS, ANTIPROLIFERATIVE ACTIVITY AGAINST CANINE CELL LINES AND EFFECT ON BIOLOGICAL MEMBRANES

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The most characteristic activity attributed to natural and synthetic compounds possessing both a lactone function and an aromatic ring is their cytotoxicity against different cancer cell lines [1]. Being interested in the synthesis of compounds exhibiting antiproliferative activity towards canine cancer lines we have synthesized recently enantiomeric β-aryl-δ-iodo-γ-lactones [2]. Encouraged by high activity of those derived from 2,5-dimethylbenzaldehyde [3] we decided to obtain also their analogs with bromine atom and evaluate their anticancer activity. Additionally, we wanted also to examine the effect of synthesized compounds on the biological membranes which are the place of first contact of the active substance with the cell.

During research enantiomeric bromolactones possesing 2,5-dimethylphenyl substituent at β-position of lactone ring have been obtained. The key step of the synthesis was a kinetic resolution of racemic (*E*)-4--(2',5'-dimethylphenyl)but-3-en-2-ol by transesterification catalyzed by lipase B from *Candida antarctica*. Having at our disposal (*R*) and (*S*)-enantiomer of this alcohol with 97 and 95% ee respectively, we were able to obtain both enantiomeric forms of desired lactones in three-step synthesis. The synthetic pathway included Johnson-Claisen rearrangement of alcohols to γ,δ-unsaturated esters followed by their hydrolysis to the corresponding acids and subsequent bromolactonization using *N*-bromosuccinimide. Finally, enantiomerically enriched γ-bromo-δ-lactones were isolated as the major products. Due to the known configuration of allylic alcohols and transfer of chirality during Johnson-Claisen rearrangement we would were able to assign the configuration of all stereogenic centers in the molecules of final lactones.

Synthesized compounds were not active against normal cell lines: mouse macrophages and fibroblasts but exhibited antiproliferative activity towards two canine cancer cell lines: B-cell lymphoma (CLBL-1) and B-cell chronic leukemia (CLB70). Their IC_{50} values ranged from 21 to 45 μg/mL and the enantiomer 4S,5S,6R was more active towards both lines than its antipode. No activity was found towards D17 cell line (canine osteosarcoma).

It has been shown that synthesized compounds do not induce hemolysis of erythrocytes, therefore they do not affect destructively on the membrane of erythrocytes. The effect on physical properties of biological membranes was be determined by the fluorimetric method using fluorescent probes (Laurdan, DPH and MC 540) located in different areas of the membrane. This research indicate that the tested lactones cause changes in the packing order of the polar heads in the lipid bilayer.

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GLYCERIN FRACTION FROM BIOESTERS MANUFACTURING AS A SUBSTRATE FOR YEAST BIOMASS PRODUCTION

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The following paper presents a concept of utilizing glycerin waste from production of Omega 3/6/9 fatty acid ethyl esters to produce microbial biomass. In the last few decades, several studies have been described that addressed the production of value-added products, such as organic acids, sugar alcohols, and single cell oil, may be obtained by yeast *Yarrowia lipolytica* when cultivated in media containing glycerin. In addition, we have found that crude glycerin from biodiesel production might be a suitable substrate for fodder yeast production by *Y. lipolytica* assuring good yield and productivity.

Ten yeasts strains of *Y. lipolytica* were used as a model organism in the production of biomass rich in protein and fatty acids:*Yarrowia lipolytica* A311, *Y. lipolytica* 1.31, K1, S5, S6, S9, S10, S11, S12 and strain S17. The glycerin waste, a main by-product of bioesters manufacture, contained (w/w) 45% of raw glycerin, 44% of fatty acids and 3–4% of ethyl esters. The initial screening of isolates for biomass production from glycerin fraction (25 g/L) was performed in the shake-flasks experiment in a buffered medium (pH 4.5).

Two strains *Y. lipolytica* S6 and *Y. lipolytica* S12 with the best biomass production ability were chosen for further studies. In the bioreactor production cultures (pH 3.75), 25 and 40 g/L of pure, raw glycerin, glycerin fraction, fatty acids from bioesters production were used in the medium as a carbon sources. The analysis of technological process parameters (biomass yield, biomass volumetric production rate) and biomass chemical composition (ash, minerals, protein and fatty acids contents and compositions) demonstrated that S6 strain of *Y. lipolytica* was the most suitable for biomass production regardless of the substrate used. The biomass yield and biomass productivity obtained with this strain reached 0.51 g/g and 1.33 g/Lh, respectively. Its application allowed obtaining 21.3 g/L of the biomass. Protein concentration in the biomass varied from 19.4 to 48.0% (w/w), depending on substrate and its initial concentration.

The results are very promising, as these findings may lead to a low-cost process of fodder yeast biosynthesis enriched in essential fatty acids from a waste generated during bioesters production.

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THE APPLICATION OF IN-HOUSE PRODUCED ENZYMES IN SECOND GENERATION BIOETHANOL PRODUCTION

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The increased demand for renewable energy sources causes a growing interest in the use of ligninocellulosic raw materials for the production of bioethanol, i.e. 2nd generation biofuel. Unfortunately, due to the relatively high production cost, liquid biofuels from lignocellulose are not widely distributed. Reducing the costs of the process can significantly contribute to increasing the production scale, and can be achieved, among others, through the use of cheap enzyme preparation, the proper raw material and the right production method. Therefore, the aim of the study was to evaluate the possibility of using an enzymes preparation obtained in the laboratory conditions for the production of bioethanol by separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) of sugars present in sweet sorghum. To compare the effects, the analogous systems of bioethanol production using the commercial preparation (Cellic® CTec2) were performed.

The cultures of *Trichoderma citrinoviride* C1 were carried out in solid medium composed of beet pulp and wheat bran (1:1) at 25°C for 10 days. The obtained post-culture extract was concentrated by ultrafiltration (10 kDa), and the enzymes preparation (named C1) in the SHF and SSF processes were used.

Sweet sorghum Sucrosorgo 506 was used as a raw material in the bioethanol production process. Sorghum plants (21% w/w, 23.96% d.m.) were treated by high temperature (150°C, 1 h) and acid hydrolysis (H_2SO_4, 2%), and after pH correction (5.0), were used for separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation. In the SHF method, the enzymatic hydrolysis was carried out with the enzyme preparations (cellulases activity of 21.5 U per 100 g of raw material) for 72 hours at 50°C on the shaker. In the process of ethanol fermentation (72h, 37°C) the industrial distillery yeast *Saccharomyces cerevisiae* SIHA®Activeyeast 6 strain DF 639 (EATON) at a concentration of 2 g d.m./kg were used. In the SSF process, analogous doses of enzymes and distillers' yeasts were used, and the process was carried out at 37°C for 144h. Glucose content after the enzymatic hydrolysis step in the SHF process and ethanol in the fermented liquids after the SHF and SSF processes was analyzed by HPLC.

The post-culture extract, obtained as a result of *T. citrinoviride* C1 cultivation, reached 2.4 U/ml of cellulolytic activity and 6.5 U/ml of xylanolytic activity. The four-fold concentration of the post-culture extract allowed to obtain an enzyme preparation with activities of 9.0 and 25.5 U/ml for cellulases and xylanases, respectively. The amount of glucose after enzymatic hydrolysis in the SHF processes, with both enzymes preparation, was on a similar level and amounted to 9.06 and 9.93 g/L for C1 and Cellic® CTec2, respectively. Higher amounts of ethanol in post-fermentation liquids were determined after the SHF process compared to SSF process (32.4 and 17.4% for C1 and Cellic® CTec2, respectively). The amount of ethanol produced with the commercial enzyme was slightly higher (increased by 1.74 and 2.14 g/L in SSF, respectively), which is promising and proves the importance to continue the studies on the optimization of the hydrolysis process conditions with the C1 preparation.

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GENERATION OF GENETICALLY MODIFIED APPLE PLANTS EXPRESSING β-1,3-GLUCANASE

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The fungal diseases remain a real threat for apple cultivation, harvesting, and storage. Traditional, widely used protection methods against fungal pathogens include biological and chemical treatment. These methods, however do not always guarantee full protection, generating at the same time additional costs and potential, negative impact on environment. The effort of constant search for better protection methods meets the steady increase of total annual production of apples in recent years, especially in Poland. Genetic engineering can be an alternative solution for traditional protection methods. Genetically modified plants expressing fungal cell wall digesting enzymes like ß-1,3-glucanase, could exhibit enhanced resistance against the most common fungal pathogens like: *Venturia, Podosphaera, Alternaria, Penicillium, Monilinia,* and *Botrytis*.

Agrobacterium-mediated transformation and subsequent regeneration via direct shoot induction protocol resulted in formation of 188 apple plant lines. The transgene integration in genome was confirmed for 76 of them. Apple lines exhibiting signs of dwarf phenotype, were rejected. Preselected 22 plants with normal phenotype were subjected to RNA analysis, which confirmed expression of introduced gene (ß-1,3-glucanase) in 30% of lines. These plants were then subjected to micropropagation protocol based on treatment with 2 ppm of thidiazuron (TDZ), and then with 1ppm of 6-benzylaminopurine and 0.1 ppm of 1-naphthaleneacetic acid. The procedure allowed for obtaining up to 122 clones from one starting plant (mean value of 49.85) and proved to be superior over standard regeneration method (without TDZ pretreatment) which gave up to 32 clones per plant (mean value of 7.6).

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IN VITRO CULTURES OF *DROSERA SPATULATA* AS A POTENTIAL SOURCE OF NAPHTHOQUINONES

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The genus Drosera (*Droseraceae*), commonly called sundew, is the largest group of carnivorous plants and consists of approximately 170 species. *Drosera spatulata* is a carnivorous species native to south-east Asia and Australia. The *Drosera* genus is a natural source of pharmacologically important compounds (e.g. naphthoquinones, flavonoids, anthocyanins, phenolic compounds) used as substrates in the production of pharmaceuticals. Droserae herba has been in use as an expectorant, diuretic and antispasmodic agent. In recent years, the bacteriostatic and antitumour activity of Drosera extracts has been reported. Carnivorous plants have become an important ornamental element in botanical garden collections. This fact, as well as the low propagation rate in their natural environment, is the reason for the *in vitro* propagation of carnivorous plants. In order to obtain a high reproduction rate we conducted an experiment on the influence of plant growth regulators (PGRs) on micropropagation of *Drosera spatulata*. The media were supplemented with various plant growth regulators ($mg \cdot dm^{-3}$) including auxin-indoleacetic acid (IAA) and cytokinins: kinetin (KIN), 6-benzylaminopurine (BAP), N^6-(2Isopentenyl)adenine (2iP) in the next combinations: ⅓MS, ⅓MS + 0.1 IAA, ⅓MS + 1.5 2 iP, ⅓MS + 1.5 kin, ⅓MS + 1.5 BA, ⅓MS + 1.5 kin + 0.1 IAA, ⅓MS + 1.5 BA + 0.1 IAA, ⅓MS + 1.5 2 iP + 0.1 IAA at 5.8 pH. Lengths of rosettes, widths of rosettes, heights of rosettes, numbers of new adventitious shoots, sizes of new plants, numbers of roots, lengths of roots, fresh weights were evaluated after six weeks of cultivation. The best results of multiplication we observed on ⅓MS + 1.5 2 iP, ⅓MS + 1.5 BA – an average of 24 to 25 plants were obtained from one explant. The addition 1.5 2 iP was favorably influenced by the size of new plants (1,5 cm) in correlation of its number. The addition to the medium 1.5 kin + 0.1 IAA had most preferably affected the on size of plants, rooting and increase the fresh weight. The number of regenerated new plants on this medium was smaller by more than half as compared to media containing BAP and 2iP.

The obtained plants were also used to identify chemical substances in rosettes and inflorescence shoots. Head-Space Solid-Phase Micro Extraction (HS-SPME) and solvent extraction techniques were applied to determined volatile constituents in those samples. As an analyzer gas chromoatography mass spectroscopy (GC-MS) aparatus was used. In rosetts and inflorescence shoots of *Drosera spatulata* different compounds in high content were identified. The predominant constituents in rosetts were plumbagin (1,4-naphtalenedione, 5-hydroxy-2-methyl) and phthiocol (1,4-naphtalenedione, 2-hydroxy-2methyl) which are derivatives of naphthoquinone. The predominant constitent in the inflorescence shoots was ascabin (benzoic acid, phenylmethyl ester). Identification of all volatile constituents was based on comparision of experimentaly obtained compound mass spectras with mass spectras available in NIST14 database. Also the experimentaly obtained retention index (RI) by Kovats was compared with RI available in the NIST WebBook and literature data.

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DEVELOPMENT OF NEW COMPOSITIONS OF PLANT MIXTURES AS A BASE OF DIETARY SUPPLEMENTS

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There are several dominant trends on the market of dietary supplements and functional foods. Polish consumers are more and more eager to reach products targeted at active people, food for people with allergies and health-promoting products. Polish consumers are becoming more and more aware that a healthy diet combined with physical activity has a positive effect not only on the appearance and well-being, but also on health. Therefore, there is a growing demand for products that improve physical performance, make it easier to lose weight or to supplement nutrient deficiencies. In addition, more often conscious consumers choose the natural products.

The aim of the work was to develop 5 mixtures composed of plant materials with functional properties, dedicated to specific groups of recipients.

The prepared mixtures were dedicated for athletes, people after chemotherapy, future mothers, people after antibiotic therapy, children with GAPS (Gut and Psychology Syndrome). The selection of plant raw materials included their composition in such way to have beneficial effects on organisms belonging to the indicated groups of recipients. The prepared plant mixtures were characterized by different extraction efficiency, antiradical activity and polyphenols content depending on the type and amount of each plant raw material in the mixture. Among the compositions dedicated to athletes, the most favorable profile was found in the mixture consisting of beetroot, rice protein, young barley and nettle. Among the compositions dedicated to people after chemotherapy, the most favorable profile was found in the mixture consisting of hemp protein, beetroot, young barley, nettle and lucuma. Among the compositions dedicated to future mothers, the most favorable profile was found in a mixture consisting of chokeberry, lentils, basil and vanilla. Among the compositions dedicated to people after antibiotic therapy, the most favorable profile was found in the mixture consisting of topinambour, lucuma, ginger, baobab and maitake. Among the compositions dedicated to children with GAPS syndrome, the most favorable profile was found in the mixture consisting of cranberry, coriander and grape seeds.

This work is a result of cooperation with Living Food Sp. z.o.o., which implements the project entitled "The development of new functional products produced on the basis of a consortium of probiotic strains, alpha-ketoglutaric acid and a vitamin-mineral complex." The work is financed by the National Center for Research and Development. No. Application for co-financing: POIR.01.01.01-00-0685/17.

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CAMPYLOBACTER JEJUNI ANTIGENS AFFECT THE VIABILITY AND MIGRATION OF HUMAN INTESTINAL EPHITELIAL CELLS

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Introduction. *Campylobacter sp.* causes in humans campylobacteriosis, which is manifested by stomach and intestine inflammation. Infections are spread by drinking unpasteurized milk, contaminated water and consumption of uncooked chicken or pork meat. The most important virulence factors of *Campylobacter sp.* are motility due to the presence of flagella, adherence and invasion of intestinal epithelial cells as well as toxins responsible for diarrhea and inflamatory response. In 30% of infected subjects campylobacteriosis may induce Gullain Barre syndrome (GBS), which is inflammatory demyelinating polyneuropathy. Campylobacteriosis is currently the most common zoonosis, but often remains undiagnosed.

Aim. To estimate using *in vitro* cellular models the cytotoxic properties of *C. jejuni* compounds (towards human intestinal epithelial cells HT-29 and the reference mouse fibroblasts L-929) and whether *C. jejuni* antigens influence the migration (regenerative) capacity of gastric barrier cells (human gastric epithelial cells AGS and L-929 cells).

Methods. The following antigen preparations of the reference strain *C. jejuni* ATCC 29428 were used: glycine extract (GE) – surface antigens; heat killed bacteria; sonicated bacterial cells (intracellular and surface antigens) and centrifuged sonicate (soluble antigens). The cytotoxic activity of *C. jejuni* components towards eukaryotic cells (HT-29, L-929) was assessed on the basis of the ability of the cells to reduce MTT (tetrazolium salt reduction assay).

The ability of the cells (AGS, L-929) to regenerate the wound was assessed by a „scratch assay". For MTT and wound heding assays the untreated cells were used as well as the cells preincubated for 24 h with studied compounds.

Results. Metabolic activity of human intestinal cells HT-29, but not mouse fibroblasts L-929 was diminished in response to *C. jejuni* compounds: GE, sonicated and heat killed bacteria in a dose dependent manner.

In a "scratch assay" *C. jejuni* GE and heat killed bacteria significantly diminished migration of human gastric AGS cells and wound heding.

Conclusion. *In vitro C. jejuni* antigens were cytotoxic to human intestinal epithelial cells HT-29 and inhibited the migration of gastric epithelial cells AGS. *In vivo* components of *C. jejuni* released during infection can effectively disrupt the structure and function of gastric and intestinal barierre of the host.

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STEREOSELECTIVE BIOREDUCTION OF β-ACETYLBUTYROLACTONE BY YEAST CULTURES IN PRESENCE OF DES

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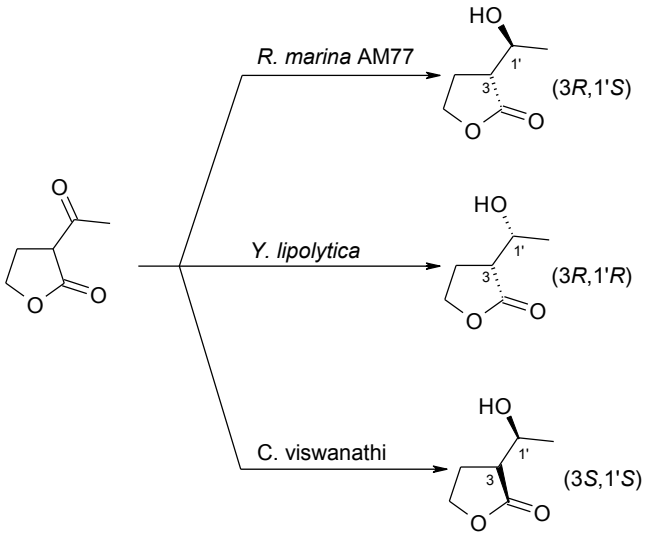
Deep eutectic solvent (DES) has been recently identified as an innovative solvent because it forms a eutectic mixture of two or more components with a melting point lower than that of both of the individual components [1]. The most popular DES in biocatalysis today is a combination of choline chloride (ChCl) and glicerol [2]. Microbial reduction of the carbonyl group is a convenient method for obtaining enantiomerically enriched β-hydroxyesters [3], what encouraged us to use a yeast strain in the biotransformation of α-acetylbutyrolactone in the presence of DES.

After one day of transformation optically pure (3R,1'R)-α'-1'-hydroxyethyl-γ-butyrolactone was obtained by means of Yarrowia lipolytica P26A in YPG medium. Only two hours was needed to obtain enantiomerically pure (3R, 1'S)-α'-1'-hydroxyethyl-γ-butyrolactone in culture of Rhodotorula marina AM77. In turn, the use of resting cells culture of Candida viswanathi AM120 in the presence of 10% DES allowed us to obtain a (3S,1'S)-enantiomer with de 85% and ee 76%.



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RESISTANCE OF *LECANICILLIUM LECANI* TO SILVER NANOPARTICLES (AGNPS)

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Lecanicillium lecani (previously described as *Verticillium lecani*) is a entomopathogenic filamentous fungus species [1]. Mainly described for its activity against aphids and whitelfly [2] but isolated from deteriorated building materials also [3]. Spores of *L. lecani* are commercially offered as anti-insect agent for plant protection since years. As example, in India 54 offers of *Verticillium lecani* suspension are presented on one www page [https://dir.indiamart.com/impcat/verticillium-lecanii.html]. To enhance the power of such preparation as biocontrol agents and prevent invasion of microbial phytopathogens the silver nanoparticles (AgNPs) could be added to *L. lecani* biomass or spores suspension.

The antimicrobial activity of AgNPs has been descried [3, 4]. Those produced by TKNano were active against bacteria, yeast and some fungi at low concentration [5]. So, the aim of the study was to check the influence of AgNPs on the growth of *L. lecani* in Bioscreen C microbial analyzer. The Bioscreen C microbial analyzer gives the opportunity to check the growth of microorganisms in the medium added of different concentrations of AgNPs at the same time, so in exactly the same conditions.

Tests of activity of AgNPs on three strains: two from German collection (DSM) and one isolated from deteriorated building materials and identified by ITS sequencing as *L. lecani,* were performed as previously described [5, 6]. Five concentrations of AgNPs were tested: 0, 3.56, 5.36, 7.14, 10.7 and 21.4 ppm.

Inhibition of three *L. lecani* strains occurred only at the highest concentration of AgNPs (21,4 ppm) and was observed during first 24 h only. So, addition of AgNPs to anti-insects preparation based on this filamentous fungus must be done at lower concentration, but still effective against some pathogenic and phytopathogenic species: *Scopulariopsis brevicalis*, *Paecilomyces variotii*, *Chaetomium globosum* and *Penicillium pinophilum* [5, 7].

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FILAMENTOUS FUNGI GROWTH INHIBITION BY SILVER NANOPARTICLES (AGNPS)

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Nowadays silver nanoparticles (AgNPs) are added as anti-microbial agent in many consumer products: chemicals, cosmetics, clothing, water filters and medical devices [1, 2]. The concentration of AgNPs needed for total inhibition of microbial growth varied from 1 to 100 ppm (mg/L), depending not only on the organisms species but on the size of particles and preparation method. AgNPs produced by TKNano were effective against bacteria, yeasts and some filamentous fungi species (*Aspergillus brasiliensis, Paecilomyces variotii, Penicillium pinophilum, Chaetomium globosum* and *Trichoderma virens)* at the concentration of 10 ppm [3]. Information on AgNPs influence on strains of other fungi genera is scarce.

The aim of the study was to determine the minimal AgNP concentration required for effective growth inhibition of *Trichoderma viride (*DMS 63065)*, Trichoderma reesei (*DSM 769)*, (*DSM 1944), *Penicillium* janthinellum (DSM 1945), *Scopulariopsis brevicalis* (DSM 9122). Fungi growth inhibition by AgNPs was tested in Bioscreen C microbial analyzer according to previously described technique [4]. Final concentration of AgNPs in culture media was : 0, 3.56, 5.36, 7.14, 10.7 and 21.4 ppm. The growth was monitored as OD during 72 h of incubation at 25°C at 20 minutes of interval.

Observed inhibition of fungal growth by AgNPs was species dependent. The most sensible to AgNPs was *T. viride* inhibited by 80% by AgNPs at concentration of 3.5 ppm of and *S. brevicalis* inhibited by 7 ppm of AgNPs. For *T. reesei* near total inhibition (90%) was observed at 10 ppm of AgNPs. In the case of *P. janthinellum* activation and inhibition of growth were observed. Growth was activated by low concentrations of AgNPs (3.56, 5.36 and 7.14 ppm), inhibited (>95%) during first 24 h at the highest tested concentration and an adaptation of fungi to AgNPs during prolonged culture (72 h) resulting in 60% inhibition was observed.

Inhibition of fungal growth by AgNPs was species dependent and was observed for the concentration of 3.56–21.4 ppm. In comparison, the concentration of 10 ppm was sufficient to inhibit the growth of majority of previously tested filamentous fungi [3,4] and 21.4 ppm only for 24 h inhibited the growth of entomopathogenic filamentous fungus *Lecanicillium lecani* [5]. In conclusion fungi species were differently influenced by AgNPs.

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PHENOLIC PHOSPHOLIPIDS AS BIOLOGICALLY ACTIVE SURFACTANTS IN PREPARATION OF LIPID NANOPARTICLES (SLN/NLC)

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Heterosubstituted phosphatidylcholine conjugates of mono- and dimethoxy derivatives of benzoic acids: veratric (PC-VERA) and *p*-anisic (PC-ANISA) has been successfully used as a new functionalized surfactants in fabrication of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). Lipid nanocarriers were prepared by the high shear homogenization/ultrasonication technique. Gelucire® 43/01 (GLCR-43) and Mygliol® 812 N (M812N) were used as a lipids whereas solution of Tween® 80 (T80) together with either 1-palmitoyl-2-(3,4-dimethoxybenzoyl)-sn-glycero-3-phosphocholine (PC-2-VERA), 1-(3,4-dimethoxybenzoyl)-2-palmitoyl-2-*sn*-glycero-3-phosphocholine (PC-1-VERA) or 1-palmitoyl-2--(4-methoxybenzoyl)-*sn*-glycero-3-phosphocholine (PC-2-ANISA) 1-(4-methoxybenzoyl)-2-palmitoyl--*sn*-glycero-3-phosphocholine (PC-1-ANISA) acts as a dispersant phase. The composition of nanocarriers formulation has been optimized using factorial design in terms of size, polydispersity and zeta potential. Optimal composition of SLN was as follows: GLCR 2%, PC 0.45%, T80 0.5%. In NLC formulation 30% of GLCR-43 was replaced with liquid lipid (M812N). Dynamic light scattering analysis showed that SLN were generally bigger than NLC with higher polydispersity. PC with methoxybenzoic acids (MBAs) residue in *sn*-2 gave always bigger particle than those with MBAs in *sn*-1 position. All nanocarriers had irregular spherical shape with size (160–230 nm), polydispersity 0.190–0.350 and negatively charged surface -|12-20| mV). Stability studies revealed that 4°C is suitable for maintaining high stability for a long term storage of prepared nanocarriers. Lumisizer® analysis showed creaming phenomenon in all nanoparticles formulation. Instability index evaluated for the centrifugation time of 3 h was in the range of 0.100–0.140 (SLN) and 0.400–0.444 (NLC) for samples stored for 7 days in 4°C whereas samples kept in 20 and 40°C showed higher instability index of ~0,4 and ~0,8 respectively. Cytotoxic effect of developed delivery systems was evaluated on human cancer epidermoid carcinoma (A431 and MeWo) using trypan blue staining and MTT assay.

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FORMULATION AND CHARACTERIZATION OF LIPID NANOCARRIERS (SLN/NLC) CONTAINING BIOLOGICALLY ACTIVE ISOMERS OF CONJUGATED LINOLEIC ACID

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Lipid nanocarriers are promising tool for delivery of biologically active compounds (BACs). In most delivery systems that have been elaborated so far BAC is applied as a component of lipid core. Here we presented a new approach in which active compound is covalently bounded with phosphatidylcholine – component of the surfactant shell. This type of delivery systems showed several advantages such as possibility of exposing ligands to the target cell receptors on the nanocarrier surface and nanoparticle compartmentation which is useful when additional BAC has to be loaded without any interaction between other active ingredients.

The present work has been carried out to study the potential of phosphatidylcholine--BAC conjugates as functionalized surfactants for fabrication of stable lipid nanocarriers (SLN and NLC). As a model compounds two homosubstituted PC with pure biological active isomers (*c*9,*t*11 and *t*10,*c*12) of conjugated linoleic acid residues were chosen. SLN and NLC consisting of Gelucire® 43/01 (GLCR-43) and Mygliol® 812 N stabilized by a mixture of Tween® 80 (T80) and either 1,2-di-(9*Z*,11*E*-octadecadienoyl-*sn*-glycero-3-phpsphocholine (PC-*c*9,*t*11CLA) or 1,2-di-(10*E*,12*Z*-octadecadienoyl)-*sn*-glycero-3--phpsphocholine (PC-*t*10,*c*12CLA) were prepared by the high shear homogenization fallowed by ultrasonication method. The mean particle size, polydispersity and zeta potential of SLN and NLC were found to be 108–110 nm, 0.150–0.180, -|13-15|mV and 110–115 nm, 0.159, -|12-15|mV respectively. Morphology was determined by TEM and revealed fairly spherical shape of nanoparticles. Both types of nanocarriers were subjected to stability study and were stored either in 4, 20 or 40°C for 7 days after that DLS and Lumisizer® analyses were performed. All samples stored in 4°C were bigger in size and polydispersity but also more stable during centrifugation. Lumisizer® analysis showed creaming phenomenon in all nanocarriers formulations. Instability index evaluated for the centrifugation time of 3 h was in the range of 0.100–0.170 (SLN) and 0.150–0.250 (NLC) for samples stored for 7 days in 4°C whereas samples kept in 20 and 40°C showed higher instability index of ~0.5–0.6.

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THE ROLE OF CYTOCHROME P-450 IN THE 4-n-NP DEGRADATION PROCESS BY ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ROBERTSI*I

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Endocrine disrupting chemicals (EDCs) are a broad category of compounds structurally similar to hormones that have ability to interfere with hormonal receptors in cells and disrupt the normal function of endocrine system in humans and animals. They are commonly occur in water, bottom sediments, air and soil. The representative of this group is 4-*n*-nonylphenol (4-*n*-NP), widely used in the production of many household articles, that easily accumulate in contaminated areas. High resistance to degradation processes due to low solubility and hydrophobic properties is characteristic for 4-*n*-NP [1, 2].

Metarhzium robertsii is a entomopathogenic fungus commonly found in soil, regulating populations of arthropods in the natural environment. It has a high ability to degrade 4-*n*-NP [3]. Analysis of 4-n-NP biodegradation products showed that *M. robertsii* decompose 4-*n*-NP especially to metabolites possess a one or two hydroxyl group next to the aromatic ring in the nonyl chain or metabolites with consecutive oxidation of the terminal methyl group of the aliphatic chain [4].

The aim of the study was to explain the role of cytochrome P-450 in 4-*n*-NP biodegradation by *M. robertsii* ARSEF 727. In the conducted research, products of biodegradation of 4-*n*-NP were analyzed using gas chromatography coupled with mass spectrometry in 24h cultures with the addition of cytochrome inhibitor, i.e. aminobenzotriazole (ABT) in 0h of incubation and with the addition of ABT after 6h of incubation.

The results of the conducted studies indicate that the role of cytochrome in the degradation of 4-*n*-NP by *M. robertsii* is crucial for the initiation of this process. It has been observed that the addition of ABT in 0h of incubation inhibited the entire pathway for *M. robertsii,* while the addition of ABT in 6h of incubation did not inhibit metabolite formation.

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MICROBIAL ELIMINATION OF PERSONAL CARE PRODUCT – METHYLISOTHIAZOLINONE

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Methylisothiazolinone (MIT, MI) is a potent biocide and preservative belonging to the isothiazolinones group. Isothiazolinones are mainly used as antibacterials in household products: cleaning products, body care products, fabric softening liquids as well as resin emulsions, paints and preparations for wood protection. Personal Care Products, like methylisothiazolinone, are a unique group of emerging environmental contaminants. The extensive use of these compounds results in their continuous release to the environment. MIT is highly toxic to aquatic organisms, which negatively affects the proper functioning of aquatic ecosystems. Therefore, it is necessary to understand the influence of this compound on the natural environment and search for microorganisms capable of its effective detoxification and elimination.

The aim of this study was finding strains of microscopic filamentous fungi capable of efficient elimination of methylisothiazolinone from the growth environment. The investigated microorganisms came from the collection of the Department of Industrial Microbiology and Biotechnology of the University of Lodz and were isolated from various polluted areas in Poland. The obtained results indicate high potential of the tested strains for MIT elimination, the rate of which was assessed using liquid chromatography tandem mass spectrometry (LC-MS / MS).

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SILICA NANOPARTICLES SYNTHESIS FROM BIOLOGICAL WASTE MATERIALS

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Nanotechnology is widely emerging field that delivers innovations and improves solutions in many areas of human activity [1]. It involves the production of nanoparticles using top-down and bottom up approaches [2]. Biotechnological protocols for nanoparticles synthesis provide an alternative with high ecological impact, especially when agro-industries residues are used as raw material [3].

The aim of presented study was the elaboration of effective strategy of rice husks (RH) biotransformation and synthesis of value-added product. The important feature of substrate used is the high content of amorphous silica. To achieve desired purpose, two fungal strains *Aspergillus parasiticus* and *Phanerochaete chrysosporium* were applied as whole-cell biocatalysts in biotransformation of plant substrate leading to Si-nanoparticles synthesis. Results were strongly dependent on the way of the biocatalysis procedure, so process conditions were optimized. SEM and EDX techniques were used to confirm the biotransformation results.

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HIGH-PROTEIN FEED COMPONENT AND ETHANOL PRODUCTION FROM SPENT BREWER'S GRAINS BY EDIBLE FILAMENTOUS FUNGI

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Spent brewer's grains (SBG) is a major solid by-product of the brewing industry which is commonly sold as a low-grade feed component. The composition of SBG, mainly containing cellulose and other non-starch carbohydrates, is responsible for its recalcitrant structure what generally limits its use as a substrate for bioprocesses. Thus, proper SBG pretreatment method including selection of catalyst and process conditions as well as selection of suitable microorganism able to utilize complex substrate matrix released are still challenging issue.

In this study SBG was utilized for simultaneous production of high-protein product and ethanol by edible fungal strains. The raw material was pretreated at mild temperature conditions (90°C) using food grade acid (H_3PO_4) and alkaline ($Ca(OH)_2$) catalysts (2 g catalyst 100 g SBG). The pretreated slurries (10% w/w dry solids) were subjected to 72 h aerobic cultivation by edible fungal strains: *Rhizopus sp., Mucor indicus* and *Neurospora intermedia* with cellulase enzyme supplementation. After fermentation, the yield and crude protein content of obtained solids (biomass with substrate residue) as well as ethanol concentration in liquid fraction of medium were determined.

It was observed that the pretreatment catalyst applied affected the final solids yield and crude protein content in the obtained products. Phosphoric acid pretreatment yielded more solid product (43.4–48.8 g kg^{-1} medium) than calcium hydroxide (37.5–41.7 g kg^{-1}). On the other hand, $Ca(OH)_2$ pretreated SBG resulted in obtaining biomass with higher protein content (up to 0.34 g g^{-1}). The highest biomass yield (48.8 g kg^{-1} medium) was obtained for H_3PO_4 pretreated SBG fermented with *N. intermedia*, while the protein content of 0.33–0.34 g g^{-1} was found in biomass obtained from $Ca(OH)_2$ treated SBG in all fungal strains applied. *N. intermedia* produced the highest amount of ethanol (3.3 g L^{-1}) amongst the fungi used. Calcium hydroxide pretreatment led to higher ethanol production by *N. intermedia* and *M. indicus* in comparison to phosphoric acid (3.3 and 2.7 versus 3.1 and 1.8 g L^{-1} respectively) while *R. sp.* yielded more ethanol when acidic pretreatment was used (2.4 versus 2.0 g L^{-1}).

This study shows the possibility of valorization of spent brewer's grains into high protein feed product together with ethanol production. The application of mild pretreatment conditions (low temperature, food grade catalyst) and edible fungus as biocatalyst seems to be an interesting option as a value addition for the brewing industry. *Neurospora intermedia* proved to be the most suitable microorganism in this case because of the highest biomass and ethanol production.

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TWO-STAGE CONTINUOUS CULTURE – TECHNOLOGY BOOSTING ERYTHRITOL PRODUCTION

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Increasing the yield of production of important chemicals may be achieved by improvement of culture parameters combined with efficient refining processes. Increased concentration of the product may be obtained by applying fed-batch, repeated batch, continuous or multistage cultures instead of simple batch culture.

The aim of the study was to enhance erythritol titer, yield and productivity from glycerol by the *Yarrowia lipolytica* Wratislavia K1 strain in a continuous two-stage chemostat culture limited by nitrogen and phosphorus. By operating the two chemostat cultures in tandem, the concentration of the carbon source and the erythritol titer increased. The aim of the second chemostat is complete utilization of the carbon from the medium, reduce the negative impact of high substrate and product concentration on the cells, provide the optimal aeration of the culture as well as maintaining the biomass concentration on high, reasonable level.

The two-stage chemostat process with glycerol resulted in 199.4 g dm^{-3} of erythritol with overall yield of 0.66 g g^{-1} and productivity of 0.8 g dm^{-3} $h^{-1.}$ These results represent almost 2.5-fold higher titer of erythritol compared to commonly used batch cultures of *Y. lipolytica* and almost 2 times higher titer and 1.3-fold increase in the product yield compared to a previously published continuous process with glycerol as a substrate.

This study showed the potential of a two-stage continuous process using a genetically unmodified strain of *Y. lipolytica* for efficient erythritol production from raw glycerol originating from different industries.

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METABOLIC ENGINEERING OF *YARROWIA LIPOLYTICA* ENHANCING THE PRODUCTION OF CITRIC ACID FROM CRUDE GLYCEROL

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Crude glycerol is a by-product of biodiesel industry, stearin production and saponification. It might contain different contaminations such as methanol or salt, thus its purification is an expensive process. However, even contaminated glycerol can act as a sufficient carbon source for the unconventional yeast *Yarrowia lipolytica*, and become a substrate for synthesis of various value-added compounds. In this work we present the metabolic engineering of *Y. lipolytica* that improves the production of citric acid from crude glycerol.

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PHOSPHATIDYLCHOLINES FUNCJONALIZED WITH 3-METHOXYBENZOIC ACID AND CLA ISOMERS AS NEW NUTRACEUTICALS

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Phenolic acids are known in the literature as biologically active compounds supplies with diet, mainly through fruit, vegetables tea and coffee. Due to the high antioxidant potential, they have become the subject of many scientific studies, as a result of which it has been proven that their high consumption is associated with lowering the risk of suffering from civilization diseases such as cancer, diabetes or cardiovascular [1]. Clifford in his review paper indicates that the daily intake of polyphenols can range from 100 mg to even 2 g and underlines that coffee is the major dietary source [1]. Unfortunately, as many other biologically active natural compounds consumed with food or in the form of oral preparations, also phenolic acids are quickly metabolizing in the human body, which significantly reduces their therapeutic potential. Recent research showed that these compounds are absorbed in the upper part of the gastrointestinal tract where they enter the liver via the portal vein and are subject to the first-pass mechanism, and in a consequence they are not observed in unchanged form in the blood and their therapeutic concentration in target tissues is impossible to achieve [2].

In order to obtain derivatives of phenolic acids characterized by increased bioavailability in biological systems, we synthesized phospholipids containing 3-methoxybenzoic acid in the *sn*-1 position. Additionally, the therapeutic activity of obtained conjugates has been increased by introducing into the *sn*-2 position of new LPC's the *cis*-9, *trans*-11/*trans*-10, *cis*-12 isomers of conjugated linoleic acid (CLA), which exhibit proven anticancer activity against the breast, intestine and liver cancer cell lines [3–5]. The presented method of modification of the structure of phenolic acids is targeted on the change the metabolism of this group of compounds in the human body. This purpose can be achieved after their lipophilization and leading to their higher concentrations in the blood expanding in this way their application in the industry.

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DECORATION OF FLAVONOIDS TOWARDS MORE BIOAVAILABLE PRODUCTS

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Flavonoids have shown promising health properties under experimental conditions; however, the low bioavailability of some must be increased for full therapeutic benefits for the prevention and treatment of disease. Therefore, further research is needed to increase the bioavailability and effectiveness of certain flavonoids, while using consumer-friendly technologies.

Most flavonoids, except catechins, are usually present in the diet as β-glycosides. The carbohydrate unit can be L-rhamnose, D-glucose, glucorhamnose, galactose, or arabinose. The sugar moiety of flavonoids is suggested as the major determinant of their absorption in humans. Studies on the bioavailability of quercetin have shown that its maximal concentration in plasma was higher and faster after intake of the glucoside than the rutinoside. These encouraging results prompted us to undertake research on other health-promoting flavonoids, which are components of dietary supplements.

Our work was focused on obtaining glucoside derivatives of flavonoids with proven biological activity; these are found in nature in relatively small amounts, so their bioavailability is of great interest. Biotransformation of flavonoids in a fungal culture yielded about 20 glucosides. We observed the influence of substituents in the B-ring of flavonoids on the regioselectivity of the glycosylation process, carried out by biocatalysts. In the near future, we plan to conduct more studies to compare the bioavailability of selected glycoside-aglycone pairs in a mouse model.

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MICROBIOLOGICAL TRANSFORMATIONS AS THE PROMISING METHOD OF PRODUCTION OF FLAVONOIDS WITH CATECHOL MOIETY

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Flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects. Many studies suggest that most of beneficial health effects associated with flavonoids intake are associated with their antioxidant capacity. In general, it is considered that a higher number of hydroxyl substituents in flavonoid molecule correlates with higher antioxidant activity. The position and the degree of hydroxylation is of primary importance in determination of an antioxidant activity. The presence of two hydroxyl groups in the *ortho* position of ring B is confirmed as the most important, although adjacent hydroxyl groups at positions C-5, C-6 and C-7 in ring A may replace ring B hydroxyl groups scavenging function.

The aim of the study was to generate the bioactive flavonoids with a catechol moiety *via* biotransformation and further comparative analysis of the antioxidant and anticancer properties between substrates and obtained products. In the course of our studies we developed a method of efficient and regioselective hydroxylation of flavonoids in the C-8 position by use the red yeasts *Rhodotorula glutinis* as biocatalysts.

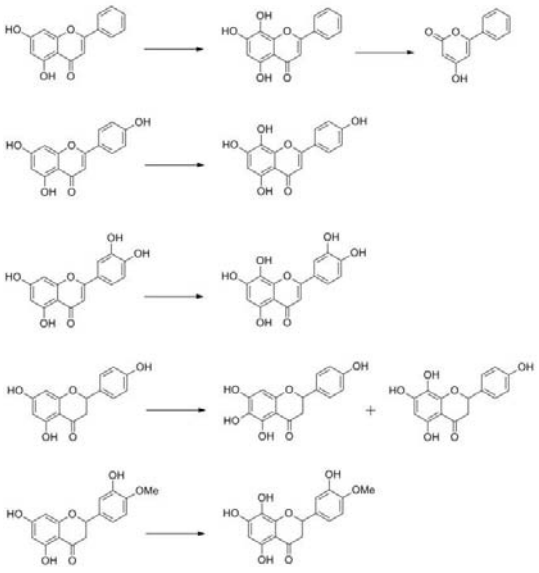

Fig. 1. Hydroxylation of flavonoids by yeast *Rhodotorula glutinis*

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MICROBIAL SYNTHESIS OF INDUSTRIALLY IMPORTANT AROMA COMPOUNDS BASED ON SOLID-STATE FERMENTATION

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Solid-state fermentation (SSF) on renewable agroindustrial side-stream products is ideal for efficient production of industrially important biocatalysts, such as lipases, proteases, cellulases, and amylases. However, there are a few literature data focused on practical application of enzymes produced by SSF.

Increasing attention is being paid to the origins of food additives, and those with natural origins are preferred. Compounds obtained by biotransformation, according to European Union and United States regulations, are regarded as natural. Due to that, interest in biotechnological production of natural-identical flavor compounds has recently increased. Researchers proposed microbial syntheses of aroma compounds based on submerged fermentation so far.

In this study, SSF was proposed as an alternative, inexpensive and environmentally friendly approach to obtain aroma compounds commonly used in food industry. Filamentous fungi, bacteria and yeasts were used for synthesis vanillin, vanillic acid and lactones (γ-decalactone, δ-decalactone, whisky lactones), utilizing oilseed cakes as a growth medium. Two different approaches were proposed. The first was based on biotransformation of precursors of these compounds. In the second route individual enantiomers of aroma compounds were obtained by kinetic resolution.

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BIOTRANSFORMATION APPLIED TO THE SYNTHESIS OF ANTIOXIDANTS

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Antioxidants are compounds having ability to neutralize free radicals- highly chemically reactive towards the living cells, resulting in many diseases. Antioxidants provide positive effect in the treatment and prevention of cancer, cardiovascular diseases, brain strokes, skin diseases and delay the process of aging. Knowledge of the destructive effect of free radicals requires a constant search for new substances that are able to prevent organism damage [1]. Biotechnological methods can be used to obtain such compounds using environmentally friendly methods which is an interesting alternative for traditional chemical methods.

The aim of the study was to use whole-cell microorganism as biocatalyst to synthesize antioxidants. It is critical to elaborate on the method of converting inexpensive starting material (2-phenylethanol) into products, such as tyrosol [2-(4-hydroxyphenyl)ethanol] and hydroxytyrosol [2-(3,4-dihydroxyphenyl) ethanol] antioxidants, in as simple a manner as possible. These compounds are known for their extraordinary antioxidant activities and are usually derived from olive oil via expensive extraction methods [2, 3]. This is why alternative methods of preparing these compounds are still under consideration.

Results indicate that biohydroxylation of 2-phenylethanol catalyzed by the *Aspergillus niger strain* mainly leads to the formation of hydroxytyrosol, while the formation of tyrosol is observed using the *Rhizopus oryzae* strain. Moreover, apply immobilization of the mycelium onto the surface of porous polyurethane foams was more effective in antioxidants production.

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HYDROLYTIC ACTIVITY OF CYANOBACTERIA – PRELIMINARY EXPERIMENTS

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Cyanobacteria are applied in biotechnology since they are a source of valuable biologically active compounds and are used for biofuels synthesis. Also, recently, these organisms are employed as biocatalysts for the synthesis of chiral, optically pure molecules, useful for many industries fields for example chemical or pharmaceutical industry. As an example – chiral alcohols are important synthetic platform in asymmetric synthesis of many different chemical structures. The hydrolytic activity of cyanobacteria have been checked for seven strains towards two model substrates. Preliminary studies allowed getting to the conclusion that the reactions are enantioselective. The following products: (R)-1-phenylethyl alcohol and (S)-1-phenylethyl alcohol were obtained as products.

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EVALUATION OF DIFFERENT EDIBLE OILS FOR α-KETOGLUTARATIC ACID PRODUCTION BY *YARROWIA LIPOLYTICA* YEATS

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α-ketoglutaric acid (KGA) is a valuable compound that is used as an active ingredient in a dietary and body-building supplements. KGA might be used in the synthesis of compounds useful in biomedicine, tissue engineering, pharmacy and as a substrate in the chemical synthesis of heterocyclic compounds. Current methods of chemical synthesis of KGA are expensive, low-yield and unfriendly to the environment, which limits the use of this acid on an industrial scale. An interesting alternative seems to be the use of biotechnological methods, particularly the microbial production of KGA with the use of *Yarrowia lipolytica* yeast. The efficient biosynthesis of KGA, which is present in the cell as an intermediate in central metabolism, is associated with the inhibition of its further transformations. In the case of *Y. lipolytica* this effect is possible to achieve by limiting the concentration of thiamine in the culture medium, since these yeasts is auxotroph against this vitamin.

The aim of the study was to examine the production of KGA by *Y. lipolytica* yeast grown on different plant oils containing media.

The process of KGA production was performed in the bioreactor, where A-8 strain of *Y. lipolytica* yeast, belonging to the Department of Biotechnology and Food Microbiology, was cultivated in the media containing edible oils (corn, linseed or sunflower) as the only source of carbon and energy. The substrate (100 g/L) was divided into five portion: 20 g/L of the oil was used at the beginning of the process whereas the rest (4 x 20 g/L of oil) was introduced into the culture at 24-hour intervals. In the mineral medium thiamine was limited at the level of 0.6 μg/L and 20% NaOH was used as the neutralizing agent in the course of biosynthesis. The process parameters were set at 30°C, aeration of 0.6 vvm, agitation of 800 rpm and pH of 4.5 with the change to 3.5 (after 24 hours of cultivation). In the samples of the culture biomass was determined gravimetrically whereas concentration KGA, citric (CA) and pyruvic acids (PA) was analyzed by HPLC method.

The strain A-8 of *Y. lipolytica* was able to grow on all the substrates under investigation, however the biomass level differ depending on kind of oil used. The highest biomass level reached 46.5 g/L and was obtained in the culture with corn oil. Application of linseed and sunflower oil resulted in the biomass concentration of 14.6 and 18.7 g/L, respectively. It was observed that kind of the substrate affected the pool of acids produced by yeast in the culture. KGA was the principal product (37 g/L with the volumetric productivity of 0.17 g/Lh) when yeast were grown on linseed oil and the concentration of PA and CA did not exceed 2.6 g/L. In turn, application of sunflower oil allowed to obtain 42.7 g/L of KGA with the volumetric productivity of 0.23 g/Lh but in the culture simultaneous formation of 18.3 g/L of CA was observed. The use of corn oil resulted in the production of 86.7 g/L of CA with volumetric productivity of 0.73 g/Lh and almost total inhibition of KGA formation (0.1 g/L), despite the fact that applied culture conditions should favor KGA synthesis.

The obtained results indicated that all the applied kind of oils were suitable for growth of *Y. lipolytica* A-8 yeast. However, their use resulted in promote production of KGA, CA or both of the acids when linseed, corn and sunflower oil was applied, respectively. The highest KGA concentration was observed when sunflower oil was applied. Interestingly, it was demonstrated that high concentration of CA might be obtained in the simple mineral medium when yeast are grown on corn oil.

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IDENTIFICATION OF MICROBIAL STRAINS ISOLATED FROM ANTARCTIC SOIL SAMPLES WITH THE CAPABILITY TO BIOPLASTIC DEGRADATION

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The accumulation of plastic waste in the environment creates an increasing ecological problem. Due to the constantly growing production of plastic, it is necessary to search for new methods of waste degradation. One of them might be the application of microorganisms showing biodegradable activity. It was shown that microorganisms have wide range of abilities and can be used in various fields. Hence, searching for microorganisms in various environments with capability to plastic degradation might be the key to solve plastic issue. Here, we show the biodegradable potential and possibilities of the microorganisms isolated from Antarctic soil samples against PLA (polylactic acid), PCL (polycaprolactone), PBS (polybutylene succinate), PBSA (polybutylene succinate adipate) and PHB (polyhydroxybutyrate). The obtained results are a good starting point for further process optimization in efficient plastic degradation.

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THE INFLUENCE OF *YARROWIA LIPOLYTICA* YEAST ON CHANGES IN THE PHYSICAL PARAMETERS OF THE HYDROCARBON – CONTAMINATED GEOLOGICAL MEDIUM AND THE GEOPHYSICAL CONTROL OF THESE CHANGES

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In the world literature, three geoelectrical models of a contaminated water-ground medium in the aeration zone are described: the high-resistivity, the low-resistivity and the so-called conceptual. High-resistivity model represents the situation where high-electrical resistivity anomalies are generated in contaminated zones. Low resistivity model – low resistivity anomalies are observed in contamination sites. The conceptual model explains these time-dependent contradictions by the process of biodegradation.

The *Yarrowia lipolytica* species (yeast) can be successfully used to remove oil-derived contaminations. They are usually a part of the preparations used to enrich the rhizosphere and improve the absorption of phosphorus. They are aerobic, non-pathogenic and considered safe and they have the ability to assimilate petroleum substances like alkanes or alkenes. Moreover, during the biochemical decomposition of hydrocarbons caused by them in the ground the organic acids are formed, which have the character of electrolyte. Therefore, their bioactivity causes that the geological medium changes its physical properties, including electrical properties and the medium becomes a high-conductivity body.

The authors carried out a series of resistivity (ERT) and conductometric (EM) tests on a place contaminated with hydrocarbons, before and after the beginning of the bioremediation process with the yeast *Yarrowia lipolytica*. The ERT measurements showed a change of anomalies from the high-resistivity to the low-resistivity and respectively the change of the anomaly of low-electrical conductivity into an anomaly of high-electrical conductivity in EM method. The high bioremediation efficiency of yeast *Yarrowia lipolytica* has been also confirmed by the laboratory tests.

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BIOTRANSFORMATION OF NARINGENIN IN THE ENZYME SYSTEM OF EDIBLE INSECTS

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Naringenin (N) is a related flavanone found in high concentrations in citrus fruits. It gives the citrus fruit its characteristic bitter taste. Reports in the literature show its antioxidant properties, antiatherosclerotic, antiinflammatory and antiulcer activity.

Biotransformation using bacteria, fungi, yeasts, and algae is an alternative tool for the structural modification of chemicals due to their high capacity to catalyze new reactions, as well as their region-selectivity and stereo-selectivity and the opportunity to receive new compounds with potential interesting biological properties. There are very few reports on the use of insect enzyme systems as biocatalysts (Marumoto et al. 2017).

In this study, we investigated the use of insects as biocatalysts for the biotransformation of N. The biocatalysts used in the study were the following insects: larvae of the millworms (*Tenebrio molitor*), zophobas (*Zophobas morio*) and madagascar cockroach (*Gromphadorhina portentosa*). The biotransformation of N by selected larvae was investigated by adding the substrate to their food and extracted the metabolites after 3–5 days from the lyophilised insects. The extracts was analyzed by HPLC and HPLC/MS.

Shinsuke Marumotoa, Yoshiharu Okuno b, Yohei Miyamotoc, Mitsuo Miyazawa. Biotransformation of (+)-(1R,2S,4R)-borneol and (−)-(1S,2R,4S)-borneol by *Spodoptera litura* (common cutworm) *larvae*. Journal of Molecular Catalysis B: Enzymatic 115 (2015) 160–167

Shinsuke Marumoto, Yoshiharu Okuno, Yuki Hagiwara, Mitsuo Miyazawa. Biotransformation of (–)-(1R,4S)-Menthone and (+)-(1S,4R)-Menthone by the Common Cutworm *Spodoptera litura Larvae. J. Oleo Sci.* (2017) 66, (8) 883-888

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XANTHOHUMOL AND NARINGENIN: ANTIOXIDANT OR PRO-OXIDANT EFFECT?

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Xanthohumol (XH) is the main prenylated flavonoid in hop (*Humulus lupulus* L.). Naringenin (N) is a related flavanone found in high concentrations in citrus fruits. These compounds are known to possess antioxidant and interesting biological pro-health activities which give the possibility of medical applications or as food nutraceuticals. The role of antioxidants in our body is to strengthen the activity of enzymatic antioxidant systems of our cells and to prevent or delay oxidative stress caused by the increase of reactive oxygen species (ROS). Thus consumption of compounds like XH and N which are antioxidant is desirable and it is associated to prevention of several diseases. But recent reports about the anti- and pro-oxidative activity of antioxidants are contradictory.

The capacity to photosensitize the generation of singlet oxygen of XH and N was evaluated through photo-oxidation of ergosterol (E) into peroxide of ergosterol (PE) as an efficient method of indirect detection of singlet oxygen (Lagunes and Trigos 2015). Obtained results showed that N is a singlet oxigen quencher (antioxidant), but XH is a photosensitazing molecule, so in certain conditions it can favor the oxidation trough singlet oxygen formation.

Lagunes I., Trigos A., Photo-oxidation of ergosterol: Indirect detection of antioxidants photosensitizers or quenchers of singlet oxygen. *Journal of Photochemistry and Photobiology B: Biology* 145 (2015) 30–34

SYMPOSIUM CONCERNING YOLKIN ON 19.06.2018 OPENING INTERNATIONAL SCIENTIFIC CONFERENCE

BIOTECHNOLOGY – RESEARCH AND INDUSTRIAL APPLICATIONS AT FACULTY OF BIOTECHNOLOGY AND FOOD SCIENCE OF WROCŁAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES

WROCŁAW – POLAND

Place of Symposium: Scientific-Didactic Center of Wrocław University of Environmental and Life Sciences (room C2); Grunwaldzki Square 24A, 50-365 Wrocław

PROGRAMME

Scientific-Didactic Center of Wrocław University of Environmental and Life Sciences (room C2); Grunwaldzki Square 24A, 50-365 Wrocław	
9.15	**Opening ceremony and welcome – Professor Józefa Chrzanowska, Professor Tadeusz Trziszka (Wrocław University of Environmental and Life Sciences)**
1.	Agnieszka Zabłocka, Ph.D and Marta Sochocka, MSc (*Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences*) – **Yolkin polypeptide complex derived from hen egg yolk – isolation, characterization and immunoregulatory activity** (9.30–10.00)
2.	Aleksandra Zambrowicz, Ph.D (*Wrocław University of Environmental and Life Sciences*) – **Yolkin polypeptide complex isolated from egg yolks of different birds species: comparative studies, and alternative methods for isolation of yolkin** (10.00–10.15)
3.	Marta Lemieszewska, MSc (*Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences*) – **Pro-cognitive properties of the immunomodulatory polypeptide complex, yolkin, from chicken egg yolk and colostrum-derived substances: analyses based on animal model of age-related cognitive deficits** (10.15-10.30)
4.	Wioletta Kazana, MSc (*Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences*) – **Yolkin – a polypeptide complex isolated from hen egg yolk and its potential neuroprotective effect** (10.30–10.45)
5.	Professor Timo Burster (*Nazarbayev University, NU School of Science and Technology*) – **Yolkin-derived proteins increase the proteolytic capacity of the serine protease cathepsin G** (10.45–11.15)
6.	Anna Dąbrowska, Ph.D (*Wrocław University of Environmental and Life Sciences*) **– Production and characteristics of recombinant peptide YGP-40** (11.15–11.30)
7.	Professor Michał Zimecki (*Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences*) Professor Bożena Obmińska (*Wrocław University of Environmental and Life Sciences*) – **Activation of immature T and B cells by yolkin** (11.30–12.15)
Discussion (12.15–12.45)	

Zabłocka A.[1], Sochocka M.[2], Sudoł M.[1], Kazana W.[1], Mitkiewicz M.[1], Macała J.[1], Polanowski A.[3], Zambrowicz A.[3]

YOLKIN POLYPEPTIDE COMPLEX DERIVED FROM HEN EGG YOLK – ISOLATION, CHARACTERIZATION AND IMMUNOREGULATORY ACTIVITY

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Introduction. The some proteins and peptides present in the egg possess antimicrobial and immunomodulatory activity. Among them the most important is the main yolk immunoglobulin – IgY. It has been shown that IgY is accompanied by an additional polypeptide fraction named yolkin.

Aim. Mechanisms activated in response to infection involve both nonspecific and specific elements of immunity, and play a key role in combating pathogens. Macrophages, in response to patogens, secrete a wide spectrum of proinflammatory factors, such as nitric oxide (NO), reactive oxygen species or cytokines. These factors are responsible for regulating the immune response, apoptosis or defense against cancer and microorganisms. Therefore the immunomodulators which are able to activate immune cells like macrophages and blood T and B lymphocytes are crucial for the development and maintenance the effective immune response to pathological factors from the environment.

Methods. BMDM bone marrow macrophages were incubated with the IgY and yolkin complex, IgY alone and yolkin obtained by size exclusion chromatography. NO level was determined by the Griess colorimetric method. Cytokine levels were determined by ELISA. iNOS level was determined by Western blot.

Results. It was shown that the tested preparations stimulate the release of nitric oxide and cytokines by BMDM macrophages, and the observed effect was dependent on the dose of the preparation. The yolkin inductor activity was significantly higher compared to the IgY and IgY and yolkin complex.

Conclusions. The properties of polypeptides present in hen's egg indicate not only their key role towards the developing chick, but also the possibility of their potential application in the therapy of immunological disorders.

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YOLKIN POLYPEPTIDE COMPLEX ISOLATED FROM EGG YOLKS OF DIFFERENT BIRDS SPECIES: COMPARATIVE STUDIES

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Introduction. The number of proteins and peptides present in the egg possess antimicrobial and immunomodulatory activity. Among them the most important is the main yolk protein: immunoglobulin Y (IgY). Recently, it has been shown that hen IgY is accompanied by an additional polypeptide fraction named yolkin. There is no information about the presence of yolkin complex in egg yolk of different birds species.

Methods. Yolkin polypeptide complex was isolated from egg yolks of goose, pigeon, duck, patridge and hen by SEC chromatography. The protein composition of yolkin samples was determined by the SDS-PAGE method. Ability to cytokine induction was determined under *ex vivo* stimulation of human whole blood cell cultures. The interleukins (TNFα, IL-6, IL-10, IL-8) were determined by ELISA test.

Results. Electrophoretic analyses revealed that the yolkin samples isolated from egg yolks of goose, pigeon, duck and patridge consists predominantly of peptides of molecular weight ranging from about 25 to 30 kDa comparable to hen yolkin complex. Yolkin samples stimulated whole blood cells to produce cytokines: TNFα, IL-6, IL-10, and IL-8. However all of them showed a bell-shaped dose-response curve. Such a dependence indicating a regulatory activity of yolkin.

Conclusion. Yolkin polypeptide complex is present in egg yolk of various species of birds. The demonstrated biological activity of the tested yolkin preparations indicates their important role in the developing chicks.

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PRO-COGNITIVE PROPERTIES OF THE POLYPEPTIDE COMPLEX FROM HEN EGG YOLK AND COLOSTRUM-DERIVED SUBSTANCES: ANALYSES BASED ON ANIMAL MODEL OF AGE-RELATED COGNITIVE DEFICITS

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Aim. The provided study based on animal model of age-related cognitive deficits has revealed the efficacy of novel polypeptide complex from hen egg yolk, named yolkin, in improving spatial and episodic memory, due to the suggested neuroprotective and immunomodulatory properties.

Methods. The experiment was carried out in rats at 6 and 12 months of age. We used two different doses of the studied specimens (based on previous comparative studies), which were administered orally and intraperitoneally. A series of behavioural tests, including Open Field Test and Novel Object Recognition Test were used to evaluate the locomotor activity and exploratory behaviour. The functioning of episodic and spatial memory in aging rats was assessed in Morris Water Maze.

Results and conclusion. The administration of yolkin gave beneficial effects, which were compared to colostrum-derived substances with acknowledged pro-cognitive action. Regarding to the latest findings, we conclude that yolkin may represent the promising feature for the prevention and treatment of aging-related neurodegenerative disorders. The study also referred to current research on mechanism of action of yolkin and colostrum-derived preparations.

Results have been published in *Archivum Immunologiae et Therapiae Experimentalis,* 2016, 64(5), pp. 425–434.

Kazana W.[1], Zambrowicz A.[2], Macała J.[1], Polanowski A.[2], Zabłocka A.[1]

YOLKIN – A POLYPEPTIDE COMPLEX ISOLATED FROM HEN EGG YOLK AND ITS POTENTIAL NEUROPROTECTIVE EFFECT

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Introduction and Aim. Polypeptide complex accompanying chicken immunoglobulin Y, yolkin possess immunoregulatory and neuroprotective activity. The level of brain-derived neurotrophic factor (BDNF), which regulates neuronal survival and outgrowth and influences synaptic plasticity is dramatically decreased in parts of the brain affected by Alzheimer's disease. This prompted us to investigate the ability of yolkin to regulate expression and secretion of BDNF.

Methods. We used rat pheochromocytoma cell line PC12 Tet On as an *in vitro* model of neuronal-like cells. Western blotting was used to determine changes in the level of intracellular BDNF protein as well as changes in phosphorylation of cAMP response element-binding protein (CREB) and mitogen-activated protein kinases (MAPK). Whereas, changes in the extracellular level of the mature BDNF protein and changes in intracellular level of cAMP molecule (cyclic adenosine monophosphate) were tested by ELISA assay. Expression of BDNF cDNA was measured by RT-PCR technique.

Results. We have found that yolkin stimulates PC12 cells to release significant amounts of mature BDNF protein, when added at concentrations higher than 10 μg/ml. Also, an increased intracellular level of pro-BDNF protein has been shown after 1 hour of incubation with yolkin. Moreover, we have demonstrated the impact of yolkin polypeptide complex on the increased level of cAMP and activation of CREB factor. In addition, the inhibition of protein biosynthesis by cycloheximide, does not affect the secretion of BDNF in response to yolkin.

Conclusions. These preliminary results indicate that yolkin might play an important role in the protection of neuronal cells through its ability to regulate the intracellular mechanisms of BDNF production and secretion. This effect is connected with the activation of cAMP/CREB – dependent signaling pathway. The regulatory effect of yolkin complex on mechanisms responsible for transport and secretion of BDNF is also considered.

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YOLKIN-DERIVED PROTEINS INCREASE THE PROTEOLYTIC CAPACITY OF THE SERINE PROTEASE CATHEPSIN G

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Neutrophils secrete serine proteases, including cathepsin G (CatG), as a first cellular immune response against pathogens. CatG secreted at the site of inflammation has several functions, for instance, degrades pathogen-derived proteins, processes chemokines and cytokines, upregulates cell surface major histocompatibility complex class I (MHC I) molecules on immune cells, and plays an important role in antigen processing in the adaptive immune response. Recently, we found that lactoferrin (LF) enhanced the proteolytic activity of CatG. Here we investigated whether yolkin, which is a polypeptide complex naturally occurring in hen's egg yolk, increases the proteolytic activity of CatG in a similar way such as LF. The data provided show that yolkin enhances CatG activity and changes the substrate selectivity of CatG, while combination of LF and yolkin inhibits the proteolytic activity of CatG. In addition, yolkin effectively reduces the cell viability of glioblastoma cell line SNB19 in a proteolytically independent manner. In conclusion, we describe novel biochemical properties of yolkin.

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PRODUCTION AND CHARACTERISTICS OF RECOMBINANT PEPTIDE YGP-40 (RYGP-40)

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Chemical composition and the properties of the individual components of the egg result from its function as a reproductive cell whose primary role is to ensure the proper development of the embryo. Especially rich reservoir of nutrients in egg is egg yolk. It is formed as emulsified system of protein-lipid complexes. It can be also a valuable source of precursor proteins for the preparation of biologically active peptides.

Vitellogenin II (VTG) is precursor of the major proteins in yolk granules (α- and β-lipovitellin, phosvitin and HDLs). This protein is a phosphoglycoprotein belonging to the family of lipid transport proteins. Vitellogenin is encoded by many genes and occurs in many animals: insects, fish and amphibians (Tufail and Takeda 2008).

VTG is not well known, but it seems to be a promising research object of potentially wide application. The recent results of research conducted by Polanowski et al. (2012) showed that the products of VTG proteolysis (known as Yolkin) accompany IgY isolated from egg yolk plasma, enhancing its biological activity. Yolkin is complex of a peptides of molecular weight ranging from 1 to about 36 kDa. It reveals immunoregulatory properties and is a potent inducer of the release of cytokines, crucial for the immune system (IL-1ß and IL-6), exceeding activity level of the reference formulation (Colostrinin).

The analysis of the sequence of N-terminal fragments of 8 purified Yolkin peptides showed that all of them were homologous to the C-terminal domain of vitellogenin II. All of these peptides show immunostimulatory activity and associate with IgY to form an active complex.

The aim of the research was obtaining the recombinant form of C-terminal fragment of VTG (peptide YGP-40) in heterologous expression system of *E. coli*, the optimalization of it's isolation and purification method of and a preliminary analysis of it's bioactivity. The nucleotide sequence of YGP-40 was obtained by the reverse translation of its protein sequence between aminoacids 1572 and 1850, with the codon bias for *E. coli* expression system. The sequence was cloned into the His-Tag pQE80L expression vector. The recombinant YGP-40 (rYGP-40) was purified form inclusuion bodies on Immobilized metal affinity chromatography (IMAC) with Ni- NTA His·Bind® Resin [Merck Millipore] and TALON® Metal Affinity Resin [Clontec]. In the obtained preparation the IL-6, Il-10 and THF alpha inducing activity was analyzed. It was observed that the rYGP-40 expresses the activity but at the lower level in comparison to yolkin isolated directly from egg yolk.

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ACTIVATION OF IMMATURE T AND B CELLS BY YOLKIN

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Preliminary experiments revealed that BALB/c mice treated intraperitoneally with yolkin stimulated the humoral immune response to sheep erythrocytes and elevated the percentage of CD19 cells in the spleen and mesenteric lymph nodes. On the other hand, the effects of yolkin on thymocyte subsets were negligible. In vitro studies, with a yolkin preparation consisting of only one 30kDa protein band, confirmed a preferential effect of yolkin on development of B cells, since in cultures of bone marrow cells yolkin at concentrations of 100, 50, 25 and 12.5ug/ml increased the content of CD19+ cells, in comparison with ovalbumin, a control protein, by 14.1, 9.8, 10.9 and 6.2%, respectively. The content of single positive CD4+ cells in thymocyte culture was increased only by about 2%.

These results prompted us to study signaling pathways in T and B cell lines, as well in resident cells in thymus, bone marrow and spleen, associated with cell activation. We determined expression of three types of MAP kinases, the extracellular signal-regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and p38. We found that yolkin in culture of Jurkat cells, an immature T cell line, strongly upregulated expression of all three MAP kinases, suggesting elicitation of signaling pathways leading to cell activation/survival.

In addition, the protein preferentially elevated expression of all MAP kinases in cultures of bone marrow cells and thymocytes, but not in splenocytes. These results suggest that the protein acts on immature B and T cells contained in the primary lymphoid organs. The preparation contains only 0.6% of phosphorus, thus it is not related to phosvitin. Further investigations on the mechanism of action of this protein fraction are underway.

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AN ALTERNATIVE METHODS FOR ISOLATION OF YOLKIN FROM HEN'S EGG YOLKS

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Introduction and aim. Although egg is a readily available high protein material and source of bioactive proteins and peptides, its use may be limited by isolation methods that are usually long-term and effective on a laboratory scale, one such example is yolkin. This is why an attempt was made to develop a method for the isolation of egg yolk-derived polypeptides that would be fast and easy-to-reproduce on an industrial scale.

Methods. The starting formulation, a complex of IgY and yolkin, was precipitated with acetone, ethanol and perchloric acid added to the final test concentrations 30–70% and 0.075–0.15M, respectively. Yolkin formulations were quantitatively and qualitatively analyzed and their ability to induce human whole blood cells to secrete interleukin -6, -10 and TNFα was determined. Results were referred to control – yolkin obtained with the use of size-exclusion chromatography. Additionally yolkin was partially fractionated with the use Zorbax GF- 250 column on HPLC system.

Results. The efficiency of isolation ranged from 9.44 to 2.13%, and with the increase in ethanol, acetone or perchloric acid concentration in the test, the yield decreased. The effectiveness of the isolation increased with the concentration of these agents in the purified material. The use of an alternative methods of isolation allowed the complete isolation of IgY from yolkin. The yolkin formulations obtained by the proposed method retained the ability to induce human blood cells to secrete cytokines. Additional fractionation of yolkin led to obtain 2 fractions (35 kDa and 25-15 kDa) differing with the presence of particular polypeptides. Results demonstrated that yolkin fractions at doses of 10 and 100 μg/ml strongly stimulated the whole blood cells to release IL-6, IL-10 and TNF-α. However unfractionated yolkin exerted the strongest cytokine inducing activity.

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