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OxiZymes in Lublin

OxiZymes in Lublin 2024

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OxiZymes in Lublin

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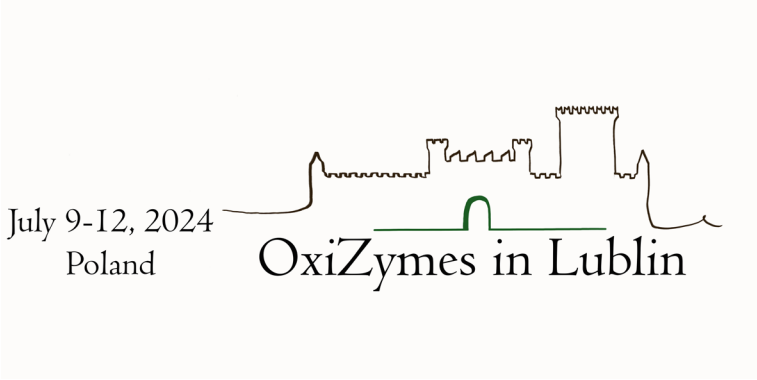
9-12 July 2024



Dear Colleagues,

It is a great pleasure to welcome you to Lublin for the 11th OxiZymes meeting on behalf of the Scientific Committee of OxiZymes and the local organizing committee.

Since the first meeting in 2002 in Cassis (France), the OxiZymes meetings have been established as a platform for scientific discussions on the mode of action of oxidoreductases. Building on the success of the previous OxiZymes meetings (Cassis, Napoli, Oeiras, Helsinki, Leipzig, Marseille, Vienna, Wageningen, Belfast, Siena), OxiZymes in Lublin 2024 will emphasize the latest development in oxidoreductases research, including their discovery, structure and function, engineering, performance, and applications.

The scientific programme will feature 34 oral presentations, including the lecture of the Sophie Vanhulle Prize. The scientific programme starts on Tuesday with the special PreConference BioToP Session as a part of the PhD programme at the University of Natural Resources and Life Sciences, Vienna, with Prof. Kiyohiko Igarashi as the invited lecturer. On Wednesday morning, the sessions of the OxiZymes meeting start and we are proud to have Prof. Gustav Berggren and Prof. Frank Hollmann as the keynote speakers as well as Prof. Wiesław Gruszecki, well-known biophysicists and Vice-Rector of the Maria Curie-Skłodowska University in Lublin.

OxiZymes 2024 is hosted by the Maria Curie-Skłodowska University in Lublin, the largest university in eastern Poland, which is celebrating its 80th anniversary this year. We hope that by listening to the talks, presenting your work, and chatting with other participants during your few days here, you will find excitement, inspiration, and encouragement for your research.

We thank our sponsors and all of you who are contributing to OxiZymes 2024. We wish you a successful scientific meeting and a pleasant stay in Lublin, the city of inspiration.

Anna Jarosz-Wilkołazka

Institute of Biological Sciences

Maria Curie-Skłodowska University in Lublin

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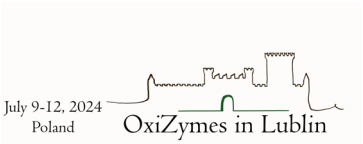

- **CONTACT PERSONS**

  If you have any questions, do not hesitate to ask the conference organizers (red-labelled badges).

- **REGISTRATION**

  Registration will be open on Tuesday, July 9th, 2024, from 15:00 to 18:00 and on Wednesday, July 10th, from 8:30.

- **POSTER INFORMATION**

  All posters have been assigned a poster number in alphabetical order of the presenting author. The numbers are indicated at the bottom of each abstract page. Posters should be mounted at the beginning of the conference and will be on display throughout the conference. The poster session with discussion will take place on Thursday, July 11, from 14:30 to 16:00. The best poster will be selected during the session, and the winner will be announced during the gala dinner.

- **SPEAKER INFORMATION**

  Speakers are asked to upload their presentation during the breaks, preferably the evening before the presentation. The duration of the oral communication is 20 minutes (15 minutes + 5 minutes of discussion). The duration of the oral communication during the BioToP Session is 15 minutes (10 minutes + 5 minutes of discussion).

- **TRANSPORTATION**

  The main means of public transportation in Lublin are buses and trolleybuses. The price of a normal ticket (ticket zone 1+2) is PLN 3.00 (15-minute ticket) or PLN 4.00 (40-minute ticket) and PLN 4.60 when bought with a credit card at a ticket machine (single-ride ticket). You must have coins or a credit card to buy a ticket from a ticket machine. It is also possible to buy a 10-day ticket for all lines (zone 1), which costs 77.00 PLN (normal) and allows unlimited transfers for 10 days from the time of validation.

  You can easily find your way using the pathfinder site https://jakdojade.pl/lublin/trasa

  WARNING: On line No 5 (from the Lublin Airport), you need a ticket for the Combined Ticket Zone (1+2); it costs 4.60 PLN (single-ride ticket).

Scientific Programme

Tuesday, 9th July 2024	
15.00-18.00	***Registration***
Pre-conference BioToP Session **Chair:** ***Dietmar Haltrich***	
16.00-16.45	REDOX SUPPORTING FOR CELLULOSE HYDROLYSIS: OXIDATIVE BOOSTING AND C1-CX THEORY ***Kiyohiko Igarashi*** *Faculty and Graduate School of Agricultural and Life Sciences, University of Tokyo, Japan*
16.45-17.00	DETANGLING BIOCHEMICAL MYSTERIES: UNDERSTANDING PYRANOSE 2- AND GLYCOSIDE 3-OXIDASES – WHAT WE KNOW SO FAR ***Anja Kostelac*** *& Dietmar Haltrich*
17.00-17.15	EXPANDING THE TOOLBOX OF ENZYMES: ALTERNATIVE FMN-DEPENDENT L-LACTATE OXIDOREDUCTASES FOR BIOSENSOR DEVELOPMENT ***Lidiia Tsvik****, Shulin Zhang, Danny O'Hare, Leander Sützl & Dietmar Haltrich*
17.15-17.45	***Coffee Break***
17.45-18.00	OXIDOREDUCTIVE ENZYMES IN THE SYNTHESIS OF LACTOBIONIC ACID ***Wiktoria Piątek-Gołda****, Justyna Sulej, Monika Osińska-Jaroszuk & Marcin Grąz*
18.00-18.15	COPROHEME DECARBOXYLASES (CHDCS); THE FINAL STEP OF HEME BIOSYNTHESIS IN GRAM-POSITIVE BACTERIA ***Gaurav Patil,*** *Hanna Michlits, Paul G. Furtmüller & Stefan Hofbauer*
18.15-18.30	SPIN-MEDIATED MYELOPEROXIDASE INHIBITION AFFECTS SUBSTRATE TURNOVER BUT NOT BINDING ***Urban Leitgeb****, Jose A. Brito, Paul G. Furtmüller, Christian Obinger & Vera Pfanzagl*

Wednesday, 10th July 2024	
8.30-10.00	***Registration***
9.00-9.20	***Opening remarks***
9.20-10.10	[FeFe] HYDROGENASE – A DIVERSE ENZYME FAMILY WITH HIGH PHOTO-BIOTECHNOLOGICAL POTENTIAL ***Gustav Berggren*** *Department of Chemistry, Uppsala University, Sweden*
Session - Oxidative and reductive biocatalysis – part 1 ***Chair: Willem van Berkel & Anna Jarosz-Wilkołazka***	
10.10-10.30	COBALT-SUBSTITUTED GLOBINS AS HYDROGEN EVOLUTION ELECTROCATALYSTS ***Gianantonio Battistuzzi,*** *Mirco Meglioli, Marco Borsari, Giulia Di Rocco, Antonio Ranieri, Carlo Augusto Bortolotti & Marco Sola*
10.30-10.50	DESIGN OF LACCASE COATED FILTRATION MEMBRANES, AS PROMISING REUSABLE BIOCATALYTIC MATERIALS FOR ENVIRONMENTAL APPLICATIONS ***Ian Coupez****, A. Wolper, Frédéric Debaste, Christine Dupont-Gillain, & Sophie Demoustier-Champagn*
10.50-11.20	***Coffee Break***

Session - Oxidative and reductive biocatalysis – part 2 ***Chair: Ligia O. Martins & Marcin Grąz***	
11.20-11.40	LIGNIN BIOMODIFICATION PLATFORM ***Sebastian Gritsch,*** *Weiß R, Mayr S, Bartolome M, Bischof S & Georg Gübitz*
11.40-12.00	PSYCHROTOLERANT *CLADOSPORIUM* SP. AS A VERSATILE BIOCATALYST FOR MONOTERPENE OXIDATION REACTIONS *Mateusz Kutyła, Marek Stankevič, Łukasz Szajnecki, Agnieszka. Świca, Edward Kozłowski, &* ***Mariusz Trytek***
12.00-12.20	SYNTHETIC APPLICATIONS IN BATCH AND FLOW OF TWO ANTI-PRELOG NAD-DEPENDENT ALCOHOL DEHYDROGENASES ***Matteo Damian****, Patrick Peters, Tanja Knaus & Francesco G. Mutti*
12.20-12.40	GENOMIC AND FUNCTIONAL DIVERSITY OF FUNGAL OXIDOREDUCTASES INVOLVED IN SUGAR METABOLISM ***Ronald P. de Vries,*** *Astrid Muelle, Jiajia Li, Mao Peng & Miia R. Mäkelä*
12.40-13.00	EXPLORING THE ROLE OF COPPER RADICAL OXIDASE FROM PATHOGENIC FUNGI ***Radka Koncitikova,*** *David Ribeaucourt, Bastien Bissaro, Yann Mathieu, Maria Cleveland, Mireille Haon, Sacha Grisel, Victor Guallar, Harry Brumer, Jean-Guy Berrin & Mickael Lafond*
13.00-14.30	***Lunch***
Session - Newly discovered oxidoreductases ***Chair: Mirjam Kabel & Grzegorz Janusz***	
14.30-14.50	NEW BACTERIAL CARBOHYDRATE OXIDASES FOR BIOTECH APPLICATIONS *André Taborda, Tomás Frazão, Ferran Sancho, Carolina Dias, João Costa, Pedro Jesus, Tiago Lopes, Cristiano Conceição, Rita Ventura, Patricia Borges &* ***Lígia O Martins***
14.50-15.10	POLYPHENOL OXIDASE ACTIVITY ON LIGNIN-UNITS ***Caio de Oliveira Gorgulho Silva,*** *Peicheng Sun, Kristian Barrett, Mark G. Sanders, Willem J.H. van Berkel, Mirjam A. Kabel, Anne S. Meyer & Jane W. Agger*
15.10-15.30	MOLECULAR DIVERSITY OF HEME-THIOLATE PEROXIDASE CLADES WITH FUNGAL AND NON-FUNGAL UNSPECIFIC PEROXYGENASES ***Marcel Zámocký*** *& Bohuš Kubala*
15.30-15.50	DISCOVERY OF NEW CAZY AA3 FAMILY OXIDOREDUCTASES FROM TREES ***Hongbo Zhao,*** *Mengyi Sun, Emma Master, Anna Kärkönen, Tanja Paasela & Maija Tenkanen*
15.50-16.15	***Coffee Break***
16.15-17.00	**Sophie Vanhulle Lecture** CHANGING THE SUBSTRATE SCOPE OF DIMERIC PYRANOSE OXIDASE FOR A GLYCOSIDE PREFERENCE THROUGH OLIGOMERIC STATE MODIFICATION ***Anja Kostelac*** *BOKU – University Natural Resources and Life Sciences of, Vienna, Austria*
17.00-17.30	LIGHT AND LIVE STRATEGIES OF PHOTOSYNTHESIZING ORGANISMS **Lecture of the Vice-Rector of Maria Curie-Skłodowska University** ***Wiesław Gruszecki***
17.30-19.00	***Welcome Cocktail and Meeting of The Scientific Committee***

Thursday, 11th July 2024	
9.00-9.50	OXYGENASES FOR MORE SUSTAINABLE CHEMISTRY – WHERE ARE WE, WHAT NEEDS TO BE DONE? ***Frank Hollmann***
Session - Structure-function relationships, protein engineering, and biomimetic approaches ***Chair: Georg Gubitz & Sergio Riva***	
9.50-10.10	BIOTIC-ABIOTIC SEMIARTIFICIAL CELLS FOR LIGHT-DRIVEN CHIRAL MOLECULE PRODUCTION ***Omer Yehezkeli,*** *Oren Bachar, Yara Zeibaq, Matan Meirovich & Yifat Cohen*
10.10-10.30	CATALYTIC AND STRUCTURAL INSIGHTS FOR THE TWO-COMPONENT INDOLE MONOOXYGENASES AND RELATED ENZYMES ***Dirk Tischler,*** *Daniel Eggerichs, Thomas Heine, Sarah Hofmann, Philipp Sowa, Julia Kratky, Renato Weiße & Norbert Sträter*
10.30-10.50	*IN VITRO* VERITAS? USING CELL-FREE PROTEIN SYNTHESIS AS AN OPTIMIZATION TOOL FOR THE SOLUBLE EXPRESSION OF AN UNSPECIFIC PEROXYGENASE IN *E. COLI* ***Ruben Walter,*** *Sophie Wardin, Jan Kiebist, & Anne Zemella*
10.50-11.20	***Coffee break***
11.20-11.40	CHARACTERIZATION AND ENGINEERING OF DYE-DECOLORIZING PEROXIDASES FROM *KITASATOSPORA AUREOFACIENS* *Enikö Hermann, Carolina F. Rodrigues, Ligia O. Martins,* ***Clemens Peterbauer*** *& Chris Oostenbrink*
11.40-12.00	ENGINEERING OF AN OXYGEN-SENSITIVE FORMATE DEHYDROGENASE ASSISTED BY A GROWTH-BASED SCREENING STRATEGY ***Feilong Li,*** *Silvan Scheller & Michael Lienemann*
12.00-12.20	COMPREHENSIVE ANALYSIS OF THE COVALENT FLAVIN IN PYRANOSE 2-OXIDASE AND PRINCIPAL COMPONENT ANALYSIS DISCOVERED THE MUTANT WITH HIGHER DEHYDROGENASE ACTIVITY THAN THE WILD-TYPE ***Yuki Yashima,*** *Taku Uchiyama, Kota Takeda, Naoki Sunagawa & Kiyohiko Igarashi*
12.20-12.40	NEW PERSPECTIVES ON METAL-PROTEIN INTERACTIONS: BLUE COPPER CENTERS, THE COUPLED DISTORTION MODEL AND THE CASE OF A GREEN CUPREDOXIN ***Giuliano Sciara,*** *M. Roger, P. Leone, N.J. Blackburn, S. Horrell, T.M. Chicano, F. Biaso, M-T. Giudici-Orticoni, L.A. Abriata, G. Hura, M. Hough & M. Ilbert*
13.00-14.30	***Lunch***
14.30-15.45	***POSTERS SESSION*** **COMPETITION FOR THE BEST POSTER**
16.00-18.00	***Tour around Lublin - City of Inspiration***
20.00	***Gala Dinner (Hotel Victoria)*** **THE BEST POSTER AWARD**

Friday, 12th July 2024	
Session - Applications of oxizymes (*fine chemistry, biorefineries, biosensors, or biomaterials)* ***Chair: Dirk Tischler & Anna Pawlik***	
9:00-9.20	LACCASES FOR DECOMPOSITION OF ENVIRONMENTAL TOXINS ***Doris Ribitsch,*** *Andreas Loibner, Wolfgang Schweiger, Gerd Schatzmayr & Georg M. Guebitz*
9.20-9.40	ENZYMATIC SYNTHESIS OF LIGNANS AND NEO-LIGNANS USING HYPERTHERMOPHILIC ENGINEERED LACCASE ***Vânia Brissos,*** *Márcia Reino, Magdalena Lejmel, Ricardo Estevinho, Maria P. Robalo, M. Rita Ventura & Lígia O. Martins*
9.40-10.00	DOMAIN MOVEMENT IN CELLOBIOSE DEHYDROGENASE IS THE BASIS OF ELECTRON TRANSFER ***Roland Ludwig,*** *Bettina Motycka, Kwankao Karnpakdee & Florian Csarman*
10.00-10.20	ARYL-ALCOHOL OXIDASES: FROM IDENTIFICATION TO BIOCATALYTIC APPLICATION ***Katja Koschorreck,*** *Nina Jankowski, Saadet Alpdağtaş & Vlada B. Urlacher*
10.20-10.40	ENGINEERING NOV1 OXYGENASE FOR HIGH-YIELDS PRODUCTION OF LIGNIN-DERIVED VANILLIN ***Mario De Simone****, Lur Alonso-Cotchico, Vânia Brissos, Maria Fátima Lucas & Lígia O. Martins*
10.40-11.00	DIRECT ELECTRON TRANSFER OF FUNGAL PYRROLOQUINOLINE QUINONE-DEPENDENT PYRANOSE DEHYDROGENASE AND ITS APPLICATION IN A BIOSENSOR ***Kota Takeda****, Kiyohiko Igarashi & Nobuhumi Nakamura*
11.00	***Closing remarks and lunch***

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[FEFE] HYDROGENASE - A DIVERSE ENZYME FAMILY WITH HIGH PHOTO-BIOTECHNOLOGICAL POTENTIAL

Gustav Berggren

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[FeFe] hydrogenases are central to the H_2 metabolism in microorganisms from all domains of life. These enzymes utilize a biologically unique organometallic cofactor, the “H-cluster”, to catalyse the interconversion of protons and molecular H_2. Due to their remarkable catalytic properties these enzymes are explored for a wide-range of biotechnological processes related to renewable energy and serve as blue-prints for the design of molecular catalysts.

Here I will present our efforts devoted to mapping out the diversity of this enzyme family [1-4] and identifying the protein features that enable their outstanding catalytic efficiencies through rational enzyme engineering [5]. Moreover, I will discuss how we explore the use of hydrogenases for photo-biotechnological H_2 production, in both photosynthetic [6,7] as well as non-photosynthetic bacteria [8-10].

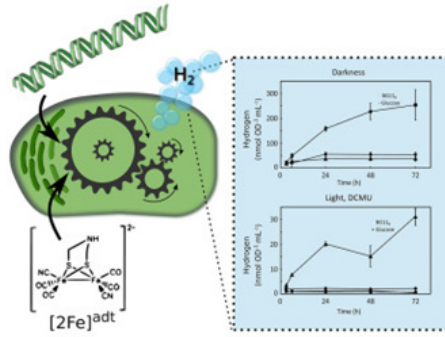

Fig. Schematic representation of the workflow to generate functional semi-synthetic [FeFe] hydrogenase in cyanobacteria. Reproduced from ref. [6].

[1] C. Greening, P. R. Cabotaje et al, Cell, accepted 2023, DOI: http://dx.doi.org/10.2139/ssrn.4520792
[2] A. Fasano, C. Baffert, C. Schumann, G. Berggren, J. A. Birrell, V. Fourmond and C. Léger, 2024, J. Am. Chem. Soc., 146, 1455-1466.
[3] H. Land, M. Senger, G. Berggren and S. T. Stripp, 2020, ACS Catalysis, 10, 7069-7086.
[4] H. Land et al, 2020, Chem. Sci., 11, 12789-12801.
[5] P. R. Cabotaje, K. Walter, A. Zamader, P. Huang, F. Ho, H. Land, M. Senger and G. Berggren, 2023, ACS Catalysis, 13, 10435-10446.
[6] A. Wegelius, H. Land, G. Berggren and P. Lindblad, 2021, Cell Reports Phys. Sci., 2, 100376.
[7] A. Wegelius, N. Khanna, C. Esmieu, G. D. Barone, F. Pinto, P. Tamagnini, G. Berggren and P. Lindblad, 2018, Energy Environ. Sci., 11, 3163-3167.
[8] M. T. Gamache, L. Kurth, D. T. Filmon, N. Plumeré and G. Berggren, 2023, Energy Advances, 2, 2085-2092.
[9] M. T. Gamache, R. Charaf, L. Kurth, D. T. Filmon, M. Senger, N. Plumeré, L. Hammarström and G. Berggren, 2023, ACS Catalysis, 13, 9476-9486.
[10] M. Lorenzi, M. T. Gamache, H. J. Redman, H. Land, M. Senger and G. Berggren, 2022, ACS Sustain. Chem. Eng., 10, 10760-10767.

OXYGENASES FOR MORE SUSTAINABLE CHEMISTRY – WHERE ARE WE, WHAT NEEDS TO BE DONE?

Frank Hollmann

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Oxygenases exhibit immense potential for creating a more sustainable chemical industry. These enzymes catalyse the selective functionalisation of inert C-H and C=C bonds, a transformation that remains a significant challenge for classical organic chemistry.

However, aside from a few applications in pharmaceutical chemistry, oxygenases currently receive limited attention in both academic and industrial research. One hypothesis is that the Oxygen Dilemma hinders or even prevents the efficient application of classical monooxygenases for large-scale (and low-cost) products. In this context, peroxygenases present a promising alternative. Nevertheless, peroxygenases are relatively new enzymes, and a considerable amount of work is required before they can be considered a viable alternative to established chemical catalysts.

In this presentation, I will provide my perspective on the current state of research, discuss the limitations, and highlight promising solutions. I hope to spark a lively discussion during the conference.

LIGHT AND LIVE STRATEGIES OF PHOTOSYNTHESIZING ORGANISMS

Wieslaw I. Gruszecki[1], Monika Zubik-Duda[1], Wojciech Grudzinski[1], Rafal Luchowski[1], Sebastian Janik[1], Karol Sowinski[1], Irena A. Pidek[2] and Radosław Dobrowolski[2]

[1]Department of Biophysics, Institute of Physics, Maria Curie-Skłodowska University, Lublin Poland; [2]Department of Geomorphology and Paleogeography, Institute of Earth and Environmental Sciences, Maria Curie-Skłodowska University, Lublin, Poland

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Most organisms living on our planet obtain energy from solar radiation. On the other hand, due to phototoxicity mechanisms, light may also be harmful to living cells. Using the examples of higher plants and algae, we will show how sophisticated and effective the precise regulation of the use of sunlight to drive biochemical reactions through photosynthesis can be.

SOPHIE VANHULLE LECTURE

CHANGING THE SUBSTRATE SCOPE OF DIMERIC PYRANOSE OXIDASE FOR A GLYCOSIDE PREFERENCE THROUGH OLIGOMERIC STATE MODIFICATION

Anja Kostelac

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Pyranose oxidase (POx) and C-glycoside oxidase (CGOx) are FAD-dependent oxidoreductases that belong to the glucose-methanol-choline superfamily and share the same sequence space [1,2]. Despite the shared overall structural fold, these two members possess homologous domains that enable (arm and head domain) or disable (insetion-1 domain and barrel shaped bottom) oligomerization [3]. In line with higher oligomerization state of POxs go a high substrate preference towards monosaccharides (such as D-glucose, D-xylose), whereas a monomeric state of CGOxs is observed in accordance with preference towards glycosides (such as homoorientin, phlorizin) [4]. In our work we aimed to engineer dimeric POx from *Kitasatospora aureofaciens* (*Ka*POx) to monomeric structure, and monomeric POx/CGOx from *Streptomyces canus* (*Sc*POx) to dimeric structure. Deletion of head and arm domain of the putative *Ka*POx resulted in enzyme variants with less hydrophobic surface, thus effecting its oligomerization. These monomeric *Ka*POx variants behaved as monomeric wild type POxs/CGOxs preferring glycosides as their substrates.

[1] T. Kumano, S. Hori, S. Watanabe, M. Kobayashi, 2022, FAD-dependent C-glycoside-metabolizing enzymes in microorganisms: Screening, characterization, and crystal structure analysis. PNAS, 118(40) e2106580118.
[2] L. Sützl, G. Foley, E.M.J. Gillam, M. Boden, D. Haltrich, 2019, The GMC superfamily of oxidoreductases revisited: analysis and evolution of fungal GMC oxidoreductases. Biotechnol Biofuels, 12, 118.
[3] Taborda A, Frazão T, Rodrigues MV, et al, 2023, Mechanistic insights into glycoside 3-oxidases involved in C-glycoside metabolism in soil microorganisms. Nat Commun,14(1), 7289.
[4] Kostelac A, Taborda A, Martins LO, Haltrich D, 2024, Evolution and separation of actinobacterial pyranose and C-glycoside-3-oxidases. Appl Environ Microbiol, 90(1), e0167623.

DETANGLING BIOCHEMICAL MYSTERIES: UNDERSTANDING PYRANOSE 2- AND GLYCOSIDE 3-OXIDASES – WHAT WE KNOW SO FAR

Anja Kostelac[1,2] and Dietmar Haltrich[1]

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The Carbohydrate-Active enZYme (CAZy) database contains catalytic modules and domains of enzymes that degrade, modify, or create glycosidic bonds. In conjunction to the CAZy enzymes, auxiliary activity enzymes provide a helping hand during lignocellulose degradation. The CAZy auxiliary activity family 3 (AA3) belongs to the glucose–methanol–choline (GMC) superfamily of FAD-dependent oxidoreductases. Subfamily AA3_4 is composed of pyranose 2-oxidases. Pyranose oxidase (POx) is an FAD-dependent oxidoreductase catalyzing preferably glucose oxidation at the C2 position [1]. *C*- and *O*-glycosides are naturally found in plants and are known to be metabolized by glycosyltransferases and glycoside hydrolases [2]. In contrast, soil bacteria can metabolize certain *C*-glycosides by a two-step reaction, with the first step being the oxidation of the sugar moiety *via* oxygen-dependent enzymes. Kumano et al. recently reported the characterization of the bacterial enzyme *C*-glycoside 3-oxidase (CGOx), catalyzing this first step of *C*-glycoside metabolism by oxidizing the C3 position of the sugar moiety [3]. Since then, a few additional CGOxs have been characterized [4,5]. In this overview presentation, I will provide the biochemical, functional, structural, and phylogenetic insights into the shared sequence space of POxs and CGOxs and their monosaccharide and *C*-glycoside metabolism. The topic of *C*- and *O*-glycoside deglycosylation will be also included in this presentation, exploring its significance as a symbiotic factor in plant-microbe interactions [6,7]. Besides providing the current knowledge, the evolutionary pathway of the mentioned sequence space will be contextualized within a broader perspective [8].

[1] C. Leitner, J. Volc, D. Haltrich, 2001, Appl Environ Microbiol, 67, 3636-3644.
[2] J. Ati, P. Lafite, R. Daniellou, 2017, Beilstein J Org Chem, 13, 1857-1865.
[3] T. Kumano, S. Hori, S. Watanabe, M. Kobayashi, 2022, PNAS, 118(40) e2106580118.
[4] Taborda A, Frazão T, Rodrigues MV, et al, 2023, Nat Commun,14(1), 7289.
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Please see the poster No 26.

EXPANDING THE TOOLBOX OF ENZYMES: ALTERNATIVE FMN-DEPENDENT L-LACTATE OXIDOREDUCTASES FOR BIOSENSOR DEVELOPMENT

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For years, L-lactate biosensor development has been limited by a lack of availability and knowledge about L-lactate-specific oxidoreductases that can be used as bio-elements in these sensors. Thus, for a long time, the field relied on one commercially available *Aerococcus viridans* L-lactate oxidase (*Av*LOx) that was applied almost exclusively in the development of new L-lactate biosensor configurations. By combining different computational approaches, we explored the diversity of naturally occurring α-hydroxy acid oxidoreductases (HAOx) and identified promising bio-elements within this family specifically suited for L-lactate biosensor applications [1].

This work unveils the biochemical properties of previously uncharacterised enzymes that are homologues to *Av*LOx and identifies a distinct class of soluble L-lactate-specific, FMN-dependent α-hydroxy acid dehydrogenases within the HAOx family. These enzymes offer a game-changing solution for second-generation biosensors, a field where *Av*LOx requires significant engineering [2,3]. Additionally, they exhibit high industrial potential, garnering substantial interest beyond scientific research.

This study showcases novel L-lactate oxidases derived from firmicutes, cyanobacteria and fungi. The novel LOxs exhibit a narrow substrate specificity towards L-lactate and oxygen, making them ideal candidates for integration into existing first-generation biosensors that are developed mainly with AvLOx. Based on the comprehensive electrochemical characterisation, seven novel lactate oxidases exhibit comparable or superior performance to *Av*LOx while offering cost-effective production. This is due to high lox gene expression even under non-optimised conditions in popular bacterial-expression systems.

Our work unlocks the vast potential of the HAOx family beyond one enzyme, *Av*LOx, paving the way for the development of advanced L-lactate biosensors. This study demonstrates the existence of diverse, ready-to-use bio-elements for existing biosensor configurations, offering the potential for significant advancements in this field.

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OXIDOREDUCTIVE ENZYMES IN THE SYNTHESIS OF LACTOBIONIC ACID

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Lactobionic acid (LBA) is a polyhydroxy acid formed by the oxidation of lactose [1]. Although chemical synthesis is still an affordable method for producing LBA, biotechnological approaches based on microorganisms or biocatalytic systems with oxidoreductive enzymes are becoming more and more popular [2]. LBA is used in medicine, the food, chemical, pharmaceutical, and cosmetic industry sectors because of its chelating, moisturizing, and antioxidant properties among others [3]. The industrial application of lactobionic acid is still limited despite its tremendous biotechnological potential because of its low efficiency, expensive synthesis costs, and occasionally restrictive legal requirements.

The aim of this study was to characterize a newly developed enzyme system consisting of a cellobiose dehydrogenase from *Phanerochaete chrysosporium* (PchCDH) and laccase from *Cerrena unicolor* (CuLAC) immobilised on three different carriers (chitosan microspheres, chitosan macrospheres and controlled porosity glass) [4,5]. We used three different crosslinking agents: genipine (GEN), glutaraldehyde (GA) and polyethyleneimine (PEI) to activate the carriers. Thin-layer liquid chromatography (TLC) was used to determine the conversion efficiency of lactose to lactobionic acid.

The obtained results show that the type of carrier used, as well as the carrier surface activator, affects both the efficiency of the immobilisation process to the carrier and lactobionic acid synthesis capacity. In all experimental variants examined, the most optimal system proved to be one in which one of the enzymes is immobilised on the carrier (PchCDH) and the other (CuLAK) is free, as this combination allows almost 100% conversion of lactose to lactobionic acid [5].

Based on this study, it is possible to conclude that fungal oxidoreductive enzymes can be successfully used for the synthesis of lactobionic acid. The obtained results showed the high biotechnological potential to produce LBA in the studied systems. Although the data presented in this report are promising, further research is needed to increase the efficiency of the lactobionic acid synthesis process.

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COPROHEME DECARBOXYLASES (CHDCS); THE FINAL STEP OF HEME BIOSYNTHESIS IN GRAM-POSITIVE BACTERIA

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For all aerobic life forms, the balanced uptake, biosynthesis, and breakdown of heme are crucial for many biological processes within a cell. Bacterial pathogens require heme, an iron-containing cofactor, to cause disease, and hemoproteins, which help in energy generation by the electron transport chain, detoxification of host immune effectors, and other processes, rely on heme for their function. Therefore, inhibition of the heme biosynthesis pathway in such pathogenic bacteria can be used as an alternative to overcome the problem of antibiotic-resistant bacteria, also referred to as superbugs. The coproporphyrin-dependent (CPD) pathway of heme biosynthesis is almost exclusively utilized by Gram-positive bacterial strains. The mammalian heme biosynthesis pathway differs significantly from the one employed by Gram-positive bacteria. Coproheme decarboxylases (ChdCs) are enzymes produced by Gram-positive bacteria involved in the coproporphyrin-dependent pathway (CPD) of heme biosynthesis. It is involved in the final step of heme biosynthesis and converts coproheme into heme *b* [1]. This conversion involves a three-propionate intermediate called monovinyl monopropionate deuteroheme (MMD), which undergoes a 90° rotation in the enzyme's active site to undergo a second decarboxylation and form heme *b* [2]. The conversion requires hydrogen peroxide as a cofactor and two oxidant equivalents to transform one coproheme into heme *b*. Using X-ray crystallography (PDB id; 6XUB, 6XUC) and site-directed mutagenesis, we identified the active site residues involved in coproheme binding and its catalysis. In *Corynebacterium diphtheriae*, the coproheme decarboxylase (*Cd*ChdC) uses histidine as a distal base to catalyze the heterolytic cleavage of hydrogen peroxide to form coproheme-Compound I which is further converted to Compound I* due to internal electron transfer [3]. A neutral tyrosine radical then cleaves off the propionate groups. Furthermore, we obtained structural snapshots of all the relevant states of *Cd*ChdC using X-ray crystallography and Cryo-EM microscopy (to be published).

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SPIN-MEDIATED MYELOPEROXIDASE INHIBITION AFFECTS SUBSTRATE TURNOVER BUT NOT BINDING

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Neutrophils are pivotal in the human innate immune response, acting by engulfing invading microbes within phagosomes, where they are neutralized using hydrolytic enzymes and reactive oxidants. A key component in this antimicrobial process is the heme enzyme myeloperoxidase (MPO), which catalyses the generation of hypohalous acids from halogens and H_2O_2 during the neutrophilic oxidative burst. These hypohalous acids effectively eliminate ingested microbes [1]. However, the notorious human pathogen *Staphylococcus aureus* has evolved a robust defence strategy against this neutrophilic assault. It produces an 8 kDa protein known as SPIN (staphylococcal peroxidase inhibitor), which binds to and effectively inhibits MPO with remarkable specificity and high affinity [2,3].

SPIN is composed of a C-terminal binding domain and an N-terminal inhibitory domain. The former rapidly binds to the enzyme surface, followed by a slower insertion of the N-terminus into the active site. This subsequent phase, though slow, is vital for the inhibitor's high affinity and is crucial for MPO inhibition. It decreases MPO activity by reducing the speed of substrate diffusion in and out of the active site through the substrate channel. As the K_m value of bromide and chloride does not change significantly upon SPIN binding, we hypothesized that the N-terminal domain does not directly affect the substrate binding site of MPO [3].

To support this hypothesis, we solved crystal structures of both the MPO-SPIN-*aureus* and MPO-SPIN-*truncated* complex (a variant of SPIN lacking the N-terminal domain), co-crystallized and/or soaked with four MPO substrates (bromide, iodide, thiocyanate and selenocyanate). All four substrates do not show any significant differences between the two complexes regarding orientation or positioning within the substrate binding site. Additionally, we identified a potential second substrate binding site located in the substrate channel. Molecular dynamics simulations of substrate binding further substantiate this. Finally, by solving the first structures of human native MPO with iodide as well as selenocyanate bound to the substrate binding site we can now accurately map the binding sites of most biological MPO substrates.

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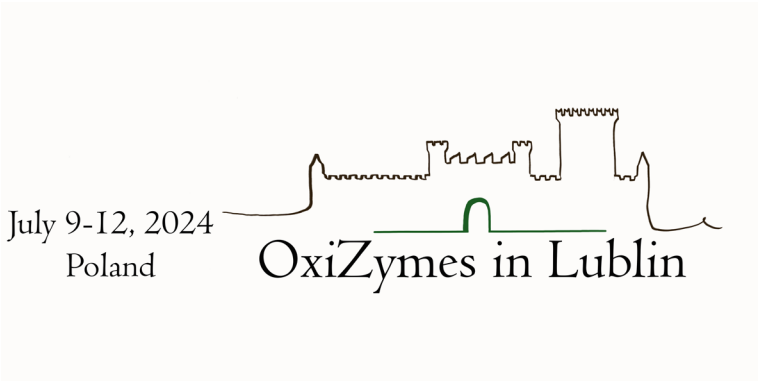
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COBALT-SUBSTITUTED GLOBINS AS HYDROGEN EVOLUTION ELECTROCATALYSTS

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Green hydrogen is the most promising alternative to fossil fuels due to its unique properties [1]. Since its production relies on the electrolytic water splitting, its application is still limited due to the low energy conversion and high costs associated to noble metal electrodes. Over the last few years, several studies have shown that cobalt coordination compounds and bioinorganic catalysts decrease the overpotential associated to H_2 evolution [2,3]. We therefore prepared the Co-substituted derivatives of two globins (myoglobin and neuroglobin), by replacing heme *b* with Co(III)-protoporphyrin IX, and used an electrochemical approach to verify their efficiency as electrobiocatalysts for molecular hydrogen evolution from aqueous protons [4]. Indeed, it turns out that, upon absorption onto pyrolytic graphite electrode, both Co-globins induce a significant lowering of the overpotential for H_2 evolution and an increase in the intensity of the corresponding electrocatalytic peak [4]. Moreover, the electrocatalytic activity is affected by protein-based acid-base equilibria and by the nature of the cobalt axial ligands [4]. The data provide some hints about the molecular determinants influencing the electrobiocatalytic mechanism of H_2 evolution.

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DESIGN OF LACCASE COATED FILTRATION MEMBRANES, AS PROMISING REUSABLE BIOCATALYTIC MATERIALS FOR ENVIRONMENTAL APPLICATIONS

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Micro-pollutants have become a worldwide issue of increasing environmental concern, with more and more toxic chemicals entering natural and human ecosystems. Most of these pollutants such as polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) or polychlorinated biphenyls (PCBs) cannot easily be removed from wastewater by conventional purification methods. Enzymatic catalysis offers a more environment-friendly option, on account of its lower energy requirements, moderate operation conditions, and non-toxic products. More precisely, laccase is a class of oxidoreductase enzymes with excellent catalytic properties and broad substrate range that meets all the conditions to become a promising option for future water purification. However, design of feasible and sustainable laccase-based wastewater treatment processes requires their immobilization onto solid material. Porous-membranes are seducing candidates as their high specific surface allows enzyme immobilization in large amount, and as they can be used as filtration material in flow reactors.

In this work, we propose to use the well-known layer-by-layer assembly surface functionalization method to successively adsorb multiple bilayers of selected polycations and of negatively-charged laccase from *Trametes versicolor* onto a solid material. Different assembly parameters were optimized on flat surface toward the formation of coatings featuring the highest activity and stability. The use of branched polyethyleneimine as polycation, and MES buffer at pH 6.5 as assembly media resulted in significantly more active multilayers. The most critical factor influencing catalytic performances was however the addition of a glutaraldehyde crosslinking step between each bilayer adsorption, resulting not only in much more stable, but also in more active coatings.

The optimized immobilization method was then used to functionalize nano-porous polycarbonate membranes. This method proved to be quite economically interesting, as more than 50% of initially introduced laccase was immobilized onto the membrane. We demonstrated that this type of membrane could successfully be used as filtration material into a flow reactor. Kinetic constants were investigated as a function of the applied flow rate. Remarkable long-term storage and operational stability make these membranes extremely promising materials for wastewater treatment processes.

To conclude, we designed highly active and stable laccase-coated filtration membranes by introducing an easy, fast and efficient immobilization strategy consisting of crosslinked polyethyleneimine and laccase bilayers. These membranes feature excellent catalytic properties as well as remarkable stability when used in flow-reactors. The evaluation of their ability to degrade pollutant is currently in progress and should allow to pave the way towards practical applications.

LIGNIN BIOMODIFICATION PLATFORM

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Despite its potential, currently less than 3% of the lignin resulting from pulp and paper production is used for value-added applications [1]. However, a number of sustainable applications have been found of enzymatically modified lignosulfonates (eLS) [2]. Though only minor structural changes are introduced in a laccase-catalysed polymerisation, these result in a drastic increase in viscosity and molecular weight [3]. While mechanical properties can be adjusted towards specific needs through addition of plasticisers [4], functionality (e.g. hydrophobicity, antimicrobial properties) can be further tuned by enzymatic coupling of functional molecules. Covalent binding of such was demonstrated on lignin model molecules using LC-MS, XPS and NMR analysis [5]. Tuned eLS have been used for development of slow release coatings for fertilisers which provide longer supply of nutrients and beneficial microbes [1] as well as in graphic paper coatings, subsidising fossil based styrene butadiene latex binders while yielding comparable properties [6]. Further on, eLS have potential as biobased glue for wood or paper-based packaging materials [7,8] and are currently investigated as binder in wood wall boards, replacing formaldehyde resins. by incorporating phase change materials, a novel bio-composite with significantly improved thermal properties will be obtained [9]. These sustainable applications demonstrate the potential of enzyme-based processes for upgrading of lignins.

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PSYCHROTOLERANT *CLADOSPORIUM* SP. AS A VERSATILE BIOCATALYST FOR MONOTERPENE OXIDATION REACTIONS

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Monoterpenes are a structurally diverse group of compounds found in the essential oils of plants. Due to the low concentration of the most valuable oxidized terpene derivatives (terpenoids), they are sought to be obtained using sustainable biocatalytic methods. Naturally occurring and inexpensive monoterpenes, such as limonene, α- and β-pinene, can be converted into value-added products *via* redox reactions using various microorganisms [1]. However, the known processes of biotransformation of monoterpenes have low efficiency due to the toxicity and volatility of these compounds and enzyme inactivation [2].

The aim of this study was to develop a novel and stable fungal biocatalyst for use in different environments for the oxidation reactions of *R*-(+)-limonene, α- and β-pinene to yield valuable products. A filamentous fungal strain isolated from Arctic soil was found to grow efficiently in the temperature range of 10-25°C and to exhibit a wide range of oxidative activity towards terpenes in mild reaction conditions. Based on the sequence analysis of the ITS1 region of the fungus, its affiliation to the genus *Cladosporium* was determined. Depending on the solvent and the biotransformation conditions, this fungus has the ability to catalyze the reactions of hydroxylation and epoxidation of terpenes. The biotransformation products were analyzed using GC-MS and NMR. In an aqueous medium, using resting mycelium in aerobic conditions at 20°C, carveol was obtained from limonene (13.1% yield). The main oxidation products of α-pinene were trans-verbenol (29.2%) and verbenone (25.6%), while those of β-pinene were trans-pinocarveol (44.7%) and pinocarveol oxide (8.9%). In an organic solvent environment (ethyl acetate, toluene), at 55°C, and in the presence of H_2O_2 and acetic acid, the freeze-dried *Cladosporium* mycelium exhibited peroxygenase activity, catalyzing the epoxidation of limonene to limonene 1,2-epoxide (57%) and limonene diepoxide (43%), and of two isomers of pinene to their respective oxides (45-78%). The reaction parameters for the epoxidation of terpenes were optimized to produce their epoxides in high yields. A mathematical model was developed to control the reaction conditions for the epoxidation of limonene, depending on the desired product, and to predict its yields over time with very high accuracy. In addition to perhydrolase activity, the mycelium also exhibited peroxygenase activity, since without the presence of acetic acid, epoxide was obtained as the product of limonene oxidation in an aqueous-organic medium.

Cladosporium mycelium is a promising catalyst for terpene oxidation due to its operational stability in organic solvents, its potential for re-use in subsequent biocatalytic cycles (>8 cycles), and the tuning of its oxidative activity to environmental conditions. Improvement of the efficiency of terpene bioconversion in an aqueous system requires further optimization of the reaction conditions to reduce the negative effects of terpenes on mycelial cells and overcome the likely inactivation of the monooxygenase system.

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SYNTHETIC APPLICATIONS IN BATCH AND FLOW OF TWO ANTI-PRELOG NAD-DEPENDENT ALCOHOL DEHYDROGENASES

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Enantioselective synthesis of alcohol is crucial for the production of active pharmaceutical ingredients, agrochemicals and fine chemicals [1]. Biocatalysis offers important tools to access these molecules with high selectivity, mainly *via* carbonyl reduction using alcohol dehydrogenases (ADHs) [2]. Unfortunately, nature offers primarily enzymes that lead to the formation of one of the two enantiomers following the Prelog rule [3]. Some anti-Prelog ADHs are known, but almost all of them are NADPH-dependent, which complicates their applicability in chemical synthesis. In this work, we disclosed two NADH-dependent anti-Prelog ADHs capable of performing the reduction of a broad range of ketones with good yields and excellent enantioselectivity [4]. Moreover, we have created two chimera enzymes in which each of the ADHs is genetically fused to a formate dehydrogenase (FDH). Thus, a single multi-functional protein is obtained that performs both the enantioselective reduction of carbonyls and the oxidation of formic acid, allowing the *in situ* recycling of the NADH cofactor. Finally, the enzyme and the cofactor have been immobilized to perform the reaction in a flow system with extremely high catalytic performance.

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GENOMIC AND FUNCTIONAL DIVERSITY OF FUNGAL OXIDOREDUCTASES INVOLVED IN SUGAR METABOLISM

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Oxidoreductases are major enzymes of fungal sugar metabolism [1]. While traditionally many of these metabolic pathways were described with single enzymes catalyzing each individual step, deletion mutants of these genes often resulted in only partial growth phenotypes, suggesting that other enzymes may also be involved. By a combination of omics and genetic analysis we demonstrated that most steps of the Pentose Catabolic Pathway (PCP) are catalyzed by three rather than a single enzyme [2]. Interestingly, these three enzymes per step were not only members of the same oxidoreductase PFAM family PF00248 but also phylogenetically closely related.

Further analysis of the phylogenetic subclade of PF00248 containing the three pentose reductases of the PCP in the industrially relevant ascomycete fungus *Aspergillus niger* revealed eight additional enzymes with no known function. Analysis of the functionality of five of these enzymes *in vitro* and *in vivo* confirmed their activity on pentoses, but also revealed significant functional differences [3].

Interestingly, the number of candidate sugar reductases differs significantly amongst fungal species, suggesting a strong evolutionary pressure towards gene duplication and gene deletion. Analysis of other pathways of fungal sugar metabolism also showed indications for enzyme redundancy, especially for steps catalyzed by oxidoreductases. Highlights of these studies will be presented.

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EXPLORING THE ROLE OF COPPER RADICAL OXIDASE FROM PATHOGENIC FUNGI

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The development of new biotechnological processes is booming and offers viable solutions for scaling up a renewable, carbon-based bio-economy. Bio-catalysis is increasingly used by industries to synthesize complex molecules, and the plant cell wall - a mixture of polysaccharides and polyaromatic polymers - represents an abundant resource that can be transformed into high-value-added products for various applications [1,2]. Fungal saprotrophs and phytopathogens naturally target plant cell wall components and produce a large panel of hydrolytic and oxidative enzymes. Therefore, fungi are currently the most important source of chemo-, regio- and stereoselective enzymes for biomass conversion. Recently, we have selected, expressed and thoroughly characterized nearly 40 novel members of the copper radical oxidase (CRO) family from auxiliary activity 5_subfamily 2 (AA5_2) of the classification of carbohydrate-active enzymes (CAZs, *www.cazy.org*) capable of oxidizing aliphatic and aromatic alcohols into their corresponding aldehydes, revealing a new reservoir of biocatalysts with high potential for green industry within the fungal kingdom [3-5]. In this context, we describe here (i) an overview of the biocatalytic diversity of AA5_2, (ii) the knowledge gained on their catalytic mechanism, structure-activity relationships affecting substrate specificity within the AA5_2 subfamily, (iii) their biological role in phytopathogens based on the existence of a natural redox partner, e.g. heme peroxidase, which is essential for pathogenic fungi, and (iv) their biocatalytic potential under applied conditions for the aroma and fragrance industry [6-10].

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NEW BACTERIAL CARBOHYDRATE OXIDASES FOR BIOTECH APPLICATIONS

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Carbohydrate oxidases represent a fascinating class of enzymes with diverse applications across various fields, from biotechnology to medicine [1]. We have been investigating bacterial pyranose oxidases (POx) that belong to the glucose-methanol-choline (GMC), and galactose oxidases (GalOx) from the copper radical oxidase (CROs) family of enzymes [2]. POXs are flavoenzymes that couple the oxidation of aldopyranose sugars, including D-glucose, at the C2 or C3 to generate keto-sugars and reduce oxygen to hydrogen peroxide. These enzymes open up new opportunities to convert carbohydrates into building blocks, bulk sweeteners, vitamins' precursors, rare sugars, and enzyme-based biosensors for sugar detection [3]. GalOx bearing to redox centers, a copper ion, and a Cys-Tyr free radical oxidizes the hydroxyl group at the C6 position of D-galactose but involves a wide range of specificities for aromatic, aliphatic, and furan-based alcohols to their corresponding aldehydes producing a variety of high-value-added products, solvents, fiber, antifreeze, and polymers.

We show that a bacterial POX oxidizes at 50,000-fold higher specificity (k_{cat}/K_m) the glucose moiety of mangiferin (a C-glycoside) to 3-keto-mangiferin and have named the enzyme glycoside 3-oxide [4] as opposed to fungal pyranose 2-oxidases that oxidize the monosaccharide D-Glc to keto-2-glucose at higher specificity. X-ray crystal structure studies and molecular dynamics simulations revealed the mechanistic features favoring catalytically competent conformational states suitable for recognition, stabilization, and oxidation of C-glycosides' glucose moiety. Furthermore, the biochemical and structural properties of a new bacterial GalOx have been explored. The enzyme contains two carbohydrate binding domains (CBMs) that have a predominant role in its thermal stabilization and activity for D-Gal-containing polysaccharides, as assessed by investigating truncated forms of the enzyme using affinity electrophoresis gels and ITC, among other approaches. The analysis of the X-ray structure recently solved at 1.2 Å, in conjunction with *in silico* studies, is helping to unveil the underlying molecular mechanisms governing its selectivity and stability. Directed evolution improved the catalytic performance not only for the canonical D-Gal substrate but also for hydroxymethylfurfural (HMF), an essential building block for biomaterials derived from cellulose and benzyl and veratryl alcohols – precursors for fragrance aldehydes upon conversion. This research contributes to advance our knowledge on CAZymes and developing cost-effective bioprocesses in lignocellulosic biorefineries.

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POLYPHENOL OXIDASE ACTIVITY ON LIGNIN-UNITS

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We present novel fungal polyphenol oxidases (PPOs) with the rare capacity to oxygenate lignin-derived guaiacyl (G) and syringyl (S)-type compounds and this carries significant prospects for lignin-related research. We expressed six PPOs from the lignocellulose-degrading ascomycetes *Myceliophthora thermophila* (Mt), *Chaetomium globosum* (Cg), and *Parascedosporium putredinis* (Pp) and investigated their reactivity towards lignin-derived compounds. All the new PPOs catalyze the *ortho*-hydroxylation of G-compounds (guaiacol, vanillic acid, and ferulic acid), forming the corresponding methoxy-*ortho*-diphenols. Remarkably, three enzymes (MtPPO7, CgPPO-1473, and CgPPO-266) are also active towards S-compounds (syringol, syringic acid, and sinapic acid) and generate the same methoxy-ortho-diphenols. Assays with $^{18}O_2$ confirm that the conversion of Scompounds occurs through the combined *ortho*-hydroxylation and *ortho*-demethoxylation of the substrate with concomitant methanol release. A coupled enzyme assay with alcohol oxidase and formaldehyde dehydrogenase allows us to confirm that the PPOs generate methanol and not formaldehyde as a co-product. The proposed PPO reaction mechanism on S units differs from those reported for aryl-O-demethylases known to date and PPOs stand out for not requiring coenzymes nor hydrogen peroxide as co-substrate. Yet, product polymerization also occurs due to the formation of reactive methoxy-*ortho*-quinones by the PPOs' diphenolase activity (two-electron oxidation). We show that the accumulation of methoxy-o-diphenols can be promoted through the addition of a reductant. The yields of diphenol vary according to the substrates' *ortho*- and *para*-substituents and the PPO variant and suggest that the monophenolase and diphenolase activities may occur *via* two discrete reaction steps or a one-step process with the same enzymes. The remarkable ability to hydroxylate and demethoxylate lignin-derived phenols carries significant implications for valorization *via* microbial or chemo-enzymatic routes.

MOLECULAR DIVERSITY OF HEME-THIOLATE PEROXIDASE CLADES WITH FUNGAL AND NON-FUNGAL UNSPECIFIC PEROXYGENASES

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The peroxidase-peroxygenase superfamily constitutes one of the four described heme peroxidase superfamilies. In the InterPro database it is annotated as IPR000028 and mainly due to contributions from numerous genomic sequencing projects it comprises already around 8,000 sequenced protein members with 98 distinct domain architectures. Although most known representatives were described from various fungal species [1] a robust phylogenetic analysis reveals that there are interesting and peculiar members also in many non-fungal sources. During our recent genomic searches we have detected the presence of numerous genes coding for unspecific peroxygenases also in several non-fungal but monocellular eukaryotic organisms. Heme-thiolate peroxidases may be present in freshwater green algae from various species of *Closterium*. This unique recently discovered HTP evolutionary clade has its closest neighborhoods among proteins originating from plant-damaging oomycetes of the genus *Phytophthora* that may have acquired the corresponding genes through a horizontal gene transfer event. Another very interesting and yet undiscovered evolutionary clade of heme-thiolate peroxidases is represented by genes originating from *Planoprotostelium fungivorum*. This mycophagous amoeba [2] is feeding on various fungi and it is interesting that also in this case the closest phylogenetic neighbors of HTP genes stem from taxonomically unrelated chytridiomycetous fungi. In our focused research we try to identify the conserved sequence motifs typical for heme-thiolate peroxidases with a peroxygenase activity. Among them most important are the highly conserved proximal amino acid triad responsible for a strong ligation of heme iron and a short sequence motif on the distal heme site responsible for the binding of another metal cation, mostly magnesium in these unspecific peroxygenases.

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DISCOVERY OF NEW CAZY AA3 FAMILY OXIDOREDUCTASES FROM TREES

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Oxidative enzymes targeting lignocellulosic substrates are presently classified into various auxiliary activity (AA) families within the Carbohydrate Active enZyme (CAZy) database [1]. Family AA3 includes FAD-containing oxidoreductases from the glucose-methanol-choline (GMC) superfamily, capable of oxidizing a wide range of carbohydrates and alcohols [2]. Oxidoreductases that efficiently utilize molecular oxygen as an electron acceptor are named oxidases, whereas those with little to no activity with molecular oxygen and prefer other electron acceptors are referred as dehydrogenases. Until now, research on AA3 oxidoreductases has primarily focused on microbial sources. Although multiple AA3-encoding genes also exist in plant genomes, plant-derived AA3 enzymes are still practically uncharacterized. As plant AA3 sequences form a phylogenetically distinct group from microbial AA3 sequences, they may encode interesting biocatalysts with novel specificities.

In the present work, a total of 25 putative AA3 protein coding gene were identified from the genome of softwood tree *Picea abies* (Norway spruce) and hardwood tree *Betula pendula* (silver birch) [3,4]. Guided by sequence analyses and the expression profiles, we selected one protein from each species for recombinant production in *Komagataella phaffii.* The substrate specificity was assessed using a recently developed high throughput screening setup [5]. The two enzymes differed significantly in their function. the spruce protein is an oxidase (*Pa*AOX1) that prefers alcohols with three carbons and showed the highest activity on glycerol, converting it to glyceraldehyde and subsequently to glyceric acid [6]. Interestingly, *Pa*AOX1 exhibited enantioselectivity, preferring L-glyceraldehyde over D-glyceraldehyde. The birch protein, identified as an aryl alcohol dehydrogenase (*Bp*ADH1), functionally resembles fungal aryl alcohol oxidoreductases. *Bp*ADH1 effectively utilized various aryl alcohols, including cinnamyl alcohol and monolignols such as coniferyl alcohol and p-coumaryl alcohol, oxidizing them to corresponding aldehydes [7]. Benzoquinone was identified as the most efficient electron acceptor, whereas oxygen was utilized at a substantially lower rate. Notably, *Bp*ADH1 also used laccase generated phenoxy radicals as electron acceptors, with the highest activity observed with monolignol-derived phenoxy radicals from ferulic acid and p-coumaric acid, while those from sinapic acid were poorly utilized. The biochemical characterization of AA3 oxidoreductases from trees provides insights into their physiological roles and offers evidence for mining tree and other plant genomes to discover novel oxidative biocatalysts.

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BIOTIC-ABIOTIC SEMIARTIFICIAL CELLS FOR LIGHT-DRIVEN CHIRAL MOLECULE PRODUCTION

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Utilizing bacteria for the production of pharmaceutical compounds or fuel is an important objective for fostering a greener and more sustainable industry. Over the last few decades, numerous biotechnological tools have emerged to tackle humanity's most pressing challenges, including climate change and disease prevention and treatment. While these engineered biological components have led to significant progress, the development of additional tools may contribute to another leap forward.

Herein, we introduce a novel concept of a self-sustained biotic-abiotic cyborg bacterium, comprising a biological system with an add-on—an inorganic nano-based organelle. Within this designed whole-cell biohybrid system, we leverage the unique structure of Stable Protein 1 (SP1) for the biosynthesis of various size-constrained inorganic nanomaterials within living systems. These nanomaterials can be coupled to biological components for utilization in diverse catalytic or photocatalytic processes [1,2].

More specifically, I will delve into the biosynthesis of CdS NPs stabilized by a pre-designed SP1 variant under ambient conditions. The resultant hybrid enables the controlled formation of crystalline nanoelements. These biohybrids can facilitate the NADPH photo-regeneration or photo-electro-regeneration, subsequently activating an imine reductase (IRED) enzyme. This photoactivation, in turn, enhances the generation of chiral amine molecules—a valuable resource in the pharmaceutical industry.

Toward the realization of these concepts, we have successfully integrated the photocatalytic NADPH regeneration system into a living bacterium. In this approach, photoactive NPs facilitate a solar-driven enzymatic cascade activation. The resulting incorporation of biosynthesized nanomaterials into living cells is anticipated to yield synergistic properties, thereby advancing current bio-nano technologies.

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CATALYTIC AND STRUCTURAL INSIGHTS FOR THE TWO-COMPONENT INDOLE MONOOXYGENASES AND RELATED ENZYMES

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Recently the flavoprotein monooxygenases (FPMOs) were reviewed according to history, biological functions and distribution, structures, mechanisms as well as applications [1]. We are especially interested in those enzymes allowing chiral epoxidation and sulfoxidation, hence we work on the so-called group E FPMOs. These enzymes are at least initiating the degradation of styrene and indole *via* a complex cascade of redox catalysis.

All group E FPMOs are two- or multicomponent-enzymes. First, NADH is used by a reductase to reduce FAD which is transferred directly or by diffusion to the epoxidase. The reduced FAD binds tightly and activates molecular oxygen for substrate oxygenation and the product is released while the formed hydroxyl-FAD decomposes to water and FAD for another cycle. This is well established for styrene monooxygenases (SMOs), but how is it in case of indole monooxygenases (IMOs). Recently, we were able to verify the epoxidation of indole substrates by an epoxide specific probe [2]. This allowed to conclude on the initial steps of indole degradation. Further, we were able to crystalize VpIndA1, the major epoxidase of IMO from *Variovorax paradoxus* EPS, in combination with various substrates and the cofactor FAD. Together with data on RoIndA1, the related enzyme from *Rhodococcus opacus* 1CP, we are now able to describe the binding of substrate and epoxidation in more detail. Those binding studies were supported by data obtained from numerous stopped-flow experiments of wildtype *Vp*IndA1 and variants. Hence, a mostly complete view on the catalysis including mechanistic details was presented for those enzymes for the first time [3]. This allowed to draw conclusions on the relation to other two-component monooxygenases. Further, the analysis of sequence-function reveals critical amino acid residues defining enantioselectivity among those and related group E FPMOs. Substrate tunnel and binding site have been identified and provided hints for protein engineering. This we used to improve epoxidation as well as sulfoxidation. Especially, *Vp*IndA1-variants are highly active sulfoxidases and produce enantiomers at high purity. But, in case of epoxidation this IMO and related enzymes are less selective which might be explained by their biological role.

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IN VITRO VERITAS? USING CELL-FREE PROTEIN SYNTHESIS AS AN OPTIMIZATION TOOL FOR THE SOLUBLE EXPRESSION OF AN UNSPECIFIC PEROXYGENASE IN *E. COLI*

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Unspecific peroxygenases (UPOs) catalyze a broad spectrum of oxyfunctionalization reactions, thus holding much promise for their use as biocatalysts in diverse biotechnological applications such as the synthesis of pharmaceuticals. While significant strides have been made in their production within fungal hosts, efforts to express them in the widely used biotechnological platform *Escherichia coli* often encounter limitations, mainly poor expression and low enzyme activity. Here, we present a cell-free approach to gain insights into the challenges hindering the heterologous expression of UPOs in *E. coli.* Cell-free protein synthesis (CFPS) harnesses the translational machinery of cell extracts in a controlled, artificial environment, offering advantages such as small-scale reactions, rapid experimentation and parallelization. Leveraging CFPS, we identified a UPO from *Podospora anserina* to be theoretically expressible in *E. coli*. Despite initial failures due to protein aggregation, we systematically investigated the impact of chemical supplements and molecular chaperones on protein solubility using CFPS. Additionally, we employed CFPS's parallelizability to engineer and evaluate mutant variants aimed at enhancing protein solubility. Incorporating these insights into heterologous expression protocols resulted in one of the highest UPO activities reported in *E. coli* to date. This work not only outlines the limitation and potential of simplified artificial systems like CFPS in overcoming challenges within complex living systems. But also shows how those artificial systems can be used to accelerate cultivation optimization.

CHARACTERIZATION AND ENGINEERING OF DYE-DECOLORIZING PEROXIDASES FROM *KITASATOSPORA AUREOFACIENS*

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Lignin is an aromatic heteropolymer recalcitrant to degradation, that is considered a possible renewable source of aromatic chemicals. In recent years more attention has been given to characterizing and applying bacterial systems for lignin utilization. Using sequence similarity searches in two genomes from the Gram-positive bacterium *Kitasatospora aureofaciens*, we identified three genes putatively encoding new dye-decolorizing peroxidase (DyP) enzymes. DyPs are hypothesized to play a role in lignin utilization and metabolization in bacteria. We biochemically characterized the three enzymes named *Ka*DyP1, *Ka*DyP2 and *Ka*DyP3, and we have found they show similar properties to previously reported bacterial DyPs. We also show that *Ka*DyP1 can perform redox cycling *in vitro* with *Ka*POx, a pyranose oxidase from the same bacterium. Using molecular dynamics (MD) simulations approaches, we also characterized the enzymes structurally. We found that *Ka*DyP2 most likely uses alternative long-range electron channeling approaches, compared to other DyPs. We also investigated the behavior of *Ka*DyP1 in the presence of 2,6-dimethoxyphenol molecules. Using site-saturation mutagenesis, we have targeted a conserved glutamate residue in loop 2 of A-type DyPs. We found variants with increased activity and decreased stability. Based on our experimental findings and simulations we propose this glutamate could act as a pH switch, which renders the enzyme fully active only in acidic environments, thus preventing cellular damage in the neutral environment of the cytoplasm [1].

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ENGINEERING OF AN OXYGEN-SENSITIVE FORMATE DEHYDROGENASE ASSISTED BY A GROWTH-BASED SCREENING STRATEGY

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Enzyme engineering is a powerful tool for improving or altering the properties of biocatalysts for industrial, research and therapeutic applications. Fast and accurate screening of variant libraries is often the bottleneck of enzyme engineering and may be overcome by growth-based screening strategies with simple processes to enable high throughput. The currently available growth-based screening strategies have been widely employed for enzymes but not yet for catalytically potent and oxygen-sensitive metalloenzymes. Here, we present a screening system that couples the activity of an oxygen-sensitive formate dehydrogenase to the growth of *Escherichia coli*. This system relies on complementation of the *E. coli* formate hydrogenlyase (FHL) complex by the Mo-dependent formate dehydrogenase H (*Ec*FDH-H). Using an *Ec*FDH-H-deficient strain, we demonstrate that growth inhibition by acidic glucose fermentation products can be alleviated by FHL complementation. This allows the identification of catalytically active *Ec*FDH-H variants at a readily measurable cell density readout, reduced handling efforts, and a low risk of oxygen contamination. Furthermore, a good correlation between cell density and formate oxidation activity was established using *Ec*FDH-H variants with variable catalytic activities. As proof of concept, the growth assay was employed to screen a library of 1032 *Ec*FDH-H variants and reduced the library size to 96 clones. During subsequent colorimetric screening of these clones, the variant A12G exhibiting an 82.4% enhanced formate oxidation rate was identified. Since many metal-dependent formate dehydrogenases and hydrogenases form functional complexes resembling the *E. coli* FHL, the demonstrated growth-based screening strategy may be adapted to enzyme components of these complexes.

COMPREHENSIVE ANALYSIS OF THE COVALENT FLAVIN IN PYRANOSE 2-OXIDASE AND PRINCIPAL COMPONENT ANALYSIS DISCOVERED THE MUTANT WITH HIGHER DEHYDROGENASE ACTIVITY THAN THE WILD-TYPE

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Introduction: Flavin is one of the most famous prosthetic groups for oxidoreductases. Some flavin-dependent proteins form a covalent bond between an amino acid and a flavin cofactor. Previous research showed that a covalent bond is lost when the amino acid attached to flavin is exchanged for another amino acid [1]. However, only a few mutations were tried before, and new covalent flavin patterns may be overlooked. To analyze the relationship between the mutation patterns at the covalent flavin and enzymatic activities, we featured a covalent flavoprotein, pyranose 2-oxidase (POx) from *Phanerochaete chrysosporium* and conducted the site-directed mutagenesis at His158, the site attached to FAD, for 19 other amino acids.

Materials and Methods: POx WT and mutants were expressed by SONIC Competent *Escherichia coli* strain BL21 (DE3) (Nippon Gene). Protein expression and the covalent FAD were identified by SDS-PAGE and UV-visible spectra. Glucose oxidase activity assay was conducted using the horse radish peroxidase method. Glucose dehydrogenase activity assay was conducted using DCPIP (dichlorophenolindophenol) as an electron acceptor and PMS (1-methoxy-5-methylphenazinium methyl sulfate) as an electron mediator. Principal component analysis of amino acids was conducted using the data of physical properties of amino acids. The color of each plot in the principal component analysis was assigned based on the effect of PMS.

Results and Discussion: All POx His158 mutants (total 19 patterns) were expressed as well as the WT. The fluorescence derived from the covalent FAD was only observed in WT in SDS-PAGE gel. Most of the His158 mutants exhibited the typical flavin spectra (two peaks around 380 nm and 450 nm) and WT showed a blue shift at 359 nm. This result corresponded to the previous works about covalent flavins [1,2]. However, two His158 mutants, H158D and H158P, did not exhibit any peaks between 350 to 450 nm. These results indicated only histidine (WT) can be attached to flavin, while His158 mutants lost the covalent bond between protein and FAD. In addition, H158D and H158P lost not only covalent linkage to FAD but also the whole of the flavin cofactor. The His158 mutants decreased glucose oxidase activity and dehydrogenase activity. The H158Y mutant, which exhibited the highest glucose oxidase activity among 19 mutants, showed only 8% of k_{cat} value compared to the WT. When dehydrogenase activity assay was conducted in 0.1 mM DCPIP, some His158 mutants, such as H158V, H158G, and H158W, increased their activities by two folds when 0.2 mM PMS were added while other His158 mutants, such as H158R and H158K, were not affected by PMS.

In the mapping of principal component analysis, two groups highly affected by PMS were found. The first one was the amino acids with small side chains. The second one was the aromatic amino acids. These trends were considered the effects of steric hindrance and π-π stacking. Then we introduce some mutations that were predicted to show higher effects of PMS. Finally, we found the L550V mutant, which exhibited 161% dehydrogenase activity compared to WT and only 86% oxidase activity compared to WT.

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NEW PERSPECTIVES ON METAL-PROTEIN INTERACTIONS: BLUE COPPER CENTERS, THE COUPLED DISTORTION MODEL AND THE CASE OF A GREEN CUPREDOXIN

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Laccases and other multicopper oxidases are multipotent enzymes of wide use in industrial biotechnologies. Their market is predicted to grow from 3 to 4.5 million USD in the period 2020-2030 [1]. These enzymes couple O_2 reduction to H_2O, performed at a quite conserved tri-nuclear copper center, to the oxidation of phenolics (and other aromatics) at a mononuclear tricoordinated, blue copper (BC) center. The latter is part of the C-teminal cupredoxin domain of laccases, a greek-key β-barrel reminiscent of the immunoglobulin fold. This domain is also responsible for the variability of laccase properties, such as low *vs* high redox potential. Our knowledge of the structure-function relationship in blue copper proteins is still in its infancy, and mononuclear, tetracoordinated cupredoxins are the perfect model proteins to dissect and understand it. They are electron transporters often found in microbial or chloroplast respiratory chains. In the last decades the Coupled Distortion Model (CDM) has been the only reference to describe the structure-function relationship in blue copper centers. This model explains the blue-to-green transition of blue copper proteins, as a consequence of metal geometry distortion from tetrahedral to tetragonal. The discovery of novel cupredoxins demonstrates their high diversity, with variations in terms of copper-binding ligands, copper center geometry, redox potential, as well as biological function. AcoP is a periplasmic cupredoxin belonging to the iron respiratory chain of an acidophilic bacterium [2]. It presents original features, including high resistance to acidic pH and a green-type copper center of high redox potential [3]. In this study [4], structural and biophysical characterization of wild-type AcoP and of two Cu-ligand mutants (H166A and M171A) confirms that the active center of AcoP is highly constrained. Comparative analysis with other cupredoxins of known structures, suggests that in AcoP the second coordination sphere might be the main determinant of active center rigidity, due to the presence of an extensive hydrogen bond network. Crystallographic structures of native reduced (1.65 Å resolution) and oxidized AcoP, confirmed by EXAFS data, unveil unusual Cu-ligand distances for a green protein, and unexpected center changes upon oxidation. These findings suggest that for AcoP the CDM might not hold valid. Finally, we show that for other cupredoxins as well, like for AcoP, the properties described do not fit well the CDM, leading us to propose that alternative models describing Cu center geometries need to be developed yet. Notably, our results seem to imply that in AcoP and other cupredoxins the second coordination sphere might affect and/or constrain the properties and geometries of Cu ligands (first coordination sphere), beyond the trajectories described by the CDM. Indirectly, they also show the rigorousness required for structural studies of metalloproteins, they give rise to some open questions on the precision of crystallographic data, and they point to structural determinants of metal properties that are progressively distant from copper (and progressively rooted within the protein matrix). Overall, our data revive the entatic state model, implying that the importance of rack-induced contributions (from the second coordination sphere) in tuning metal properties might have been, but should not be, underestimated.

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LACCASES FOR DECOMPOSITION OF ENVIRONMENTAL TOXINS

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Laccases have attracted increasing attention in decomposition of environmental toxins present e.g. in food and feed products or in contaminated soils. However, despite their relatively wide range of substrates we will show that only few laccases can attack certain petroleum hydrocarbons and mycotoxins. Three potential novel laccases were identified in an enrichment culture from soil historically contaminated with highly persistent hydrocarbons and recombinantly expressed in *E. coli*. Treatment of soil with one of the identified laccases, namely the PlL from *Parvibaculum lavamentivorans*, resulted in a significantly higher reduction of total petroleum hydrocarbons in the sample (83% w/w) compared to the microbial control (74% w/w). Hereby, PlL was especially effective in degrading hydrocarbons >C17. Remarkably, PlL was even able to reduce the residual concentration of ultra-stable diamondoids such as adamantane and diamantine by around 40% and 60%, respectively, within eight days [1]. Aflatoxins are cancerogenic secondary metabolites produced by several *Aspergillus* species. A number of agricultural relevant crop species such as corn and peanuts are common hosts to these fungi, leading to frequent contaminations throughout the globe. Strategies to remove AFB1 from the food and feed chain have been studied extensively, however, neither these approaches are cost-effective, nor they are well applicable in industry. Among several laccases tested, the recombinantly produced and purified *Bacillus subtilis* CotA (*Bs*CotA) laccase was found to decompose AFB1 without the use of mediators. At a relatively high concentration of 500 µg /L, AFB1 was decomposed at 10.75 µg/Lh at a dosage of 0.2 µM *Bs*CotA. AFQ1 and epi-AFQ1 were identified as the initial oxidation products according to mass spectrometry. Finally, genotoxicity of the formed AFB1 was assessed in several dilutions based on the de-repression of the bacterial SOS response to DNA damage indicating about 80-times reduction of toxicity when compared to AFQ1 [2].

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ENZYMATIC SYNTHESIS OF LIGNANS AND NEO-LIGNANS USING HYPERTHERMOPHILIC ENGINEERED LACCASE

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Lignin is the second most abundant polymer next to cellulose and by far the largest renewable source of aromatic building blocks on the planet. Enzymatic conversion of phenolic platform chemicals offers a promising, eco-friendly approach to effectively valorize lignin bio-wastes the pulp and paper industry boasts an annual lignin production of approximately 50 million tons, but only around 1 million tons enter the chemicals market [1]. The current challenge is to develop economically viable, waste-minimizing processes that comprehensively utilize lignin, transforming it into a sustainable feedstock for drop-in chemicals, polymers, and emerging functional materials [2].

Laccases are biocatalysts with immense potential in lignocellulose biorefineries to valorize emerging lignin monomers into environmentally friendly chemicals and materials [3]. Although the costs of enzymes have significantly decreased during the past two decades, enzymes remain a substantial cost factor in biorefining. Protein engineering helps to tailor enzymes to increase their catalytic efficiency. In the context of lignocellulose biorefinery, higher temperatures are often desirable to enhance lignin raw materials solubility and accelerating reaction rates, making thermostable enzymes promising candidates for this application.

In this study, we utilized directed evolution techniques to improve > 400-fold the catalytic efficiency (k_{cat}/K_m) of a hyper thermostable bacterial laccase [4] for syringol, a lignin-related phenolic compound. Our results demonstrated that this evolved variant showed improved oxidative activity for hydroxycinnamyl alcohols, cinnamic acid and vanillyl derivatives, indicating its versatility for various biotechnological applications. Our method proves efficient, cost-effective, green, and sustainable in obtaining biologically active (neo) lignans from lignin-phenolics compounds, such syringaresinol, pinoresinol or diapocynin, with very good to excellent yields (60%-100%), in most cases as single products, showing clear advantages over previously reported synthetic methods. These (neo)lignans hold potential applications in medicinal chemistry and polymer synthesis. They are natural plant secondary metabolites, typically extracted from plants, in time-consuming processes, underscoring the efficiency of our new enzymatic approach.

Overall, this study contributes to the development of green and sustainable bioprocesses, using robust and efficient biocatalysts to allow the full implementation of lignin as a sustainable starting material for producing high-value products.

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DOMAIN MOVEMENT IN CELLOBIOSE DEHYDROGENASE IS THE BASIS OF ELECTRON TRANSFER

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The hemoflavoenzyme cellobiose dehydrogenase (CDH) is a central part of the fungal exometabolism in lignocellulose degradation, forming the only known extracellular electron transfer chain to lytic polysaccharide monooxygenase [1]. Its function as an electron donor is not only of high physiological relevance, but has also been applied in biosensor and biofuel cell research. The basis of its well-known direct electron transfer properties has been elucidated by protein engineering, X-ray crystallography, small angle X-ray scattering, stopped-flow spectroscopy and electrochemical methods. CDH consists of a catalytic flavodehydrogenase domain and an electron transferring cytochrome domain. Both domains are connected by a flexible linker [2]. The high mobility of the cytochrome domain is the governing factor for the interdomain electron uptake from the catalytic domain, which is influenced by pH, ion concentration and activators. The interprotein electron transfer to lytic polysaccharide monooxygenase or the direct electron transfer to an electrode surface also depends on the mobility of the cytochrome domain, which is regulated by the length of the flexible interdomain linker in addition to the steric and electrostatic complementarity of the interface [3]. The complex influences on domain mobility also play a role in redox polymers used in biosensors and biofuel cells. Here, electron hopping from cytochrome domain to cytochrome domain provides an avenue for electron transport from distant enzymes to the electrode, increasing the achievable current density of the bioelectrocatalytic devices [4]. The combination of these studies demonstrates an intriguing balance of affinity and mobility of the cytochrome domain and provides suggestions for further improvement of biocatalytic and bioelectrocatalytic applications of CDH.

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ARYL-ALCOHOL OXIDASES: FROM IDENTIFICATION TO BIOCATALYTIC APPLICATION

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Aryl-alcohol oxidases (AAOs) are FAD-containing fungal oxidoreductases that catalyze the oxidation of primary aromatic or allylic alcohols to the corresponding aldehyde with the concomitant reduction of O_2 to H_2O_2. These enzymes have attracted increasing attention for application as biocatalysts in production of bio-based precursors for plastics, bioactive compounds, and flavors and fragrances [1]. However, their use is hampered by their difficult heterologous expression, often suffering from low yields [2]. As a consequence, the number of investigated AAOs and the knowledge about their substrate scope and physical properties are rare, limiting their applicability.

We have expanded the toolbox of recombinant AAOs by establishing the heterologous expression of two new AAOs in the methylotrophic yeast *Pichia pastoris* (recently reclassified as *Komagataella phaffii*) at up to 750 mg/L scale [3,4]. Biochemical investigation of PeAAO2 from *Pleurotus eryngii* P34 and MaAAO from *Moesziomyces antarcticus* revealed that both enzymes are promising biocatalysts, pH and thermostable, and convert a broad range of substrates with high activity. Sequence alignment of *Pe*AAO2 with the non-expressible *Pe*AAO1 from *P. eryngii* ATCC 90787 showed, that both enzymes differ in only 7 amino acids in the mature protein. By protein engineering, the PeAAO1 variant containing two neighbouring amino acid exchanges K583E and Q584R was generated, that showed even higher expression level than wildtype *Pe*AAO2 [5].

Further, we developed an agar plate assay for fast screening of *Pichia pastoris* mutant libraries constructed for improved activity towards any target substrate [6]. AAO expression and substrate oxidation can be easily visualized by a colorimetric reaction on agar plate without laborious cultivation in liquid medium, thereby reducing costs and materials needed.

The biotechnological potential of *Pe*AAO2 was demonstrated by the biocatalytic production of e.g. the fragrance compound piperonal from piperonyl alcohol at preparative scale [7]. *Ma*AAO was successfully applied in a two-enzyme cascade reaction together with the glyoxal oxidase *Tv*GLOX from *Trametes versicolor* for synthesis of the bioplastics precursor 2,5-furandicarboxylic acid (FDCA) from 5-hydroxymethylfurfural (HMF) [8]. In this two-enzyme system almost complete conversion of 8 mM HMF to FDCA was achieved within 24 h by continuous reactivation of *Tv*GLOX.

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ENGINEERING NOV1 OXYGENASE FOR HIGH-YIELDS PRODUCTION OF LIGNIN-DERIVED VANILLIN

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NOV1 from *Novosphingobium aromatocivorans* is a non-heme iron-dependent dioxygenase that catalyze the cleavage of C=C bonds of stilbenoid and phenylpropanoid compounds. This enzyme represents a promising and sustainable solution to chemical synthesis vanillin and simultaneously valorize lignin bio-wastes, since it is able to convert in one-step isoeugenol, an intermediate of lignin degradation, into vanillin, the most common fragrance worldwide.

Rational design near the active site led to the identification of the S283F variant that exhibited a 2-fold increase in activity and a 20-fold higher stability compared to the wild-type [1]. Biochemical and molecular dynamics experiments reveal that S283F replacement enhances the stabilization of the iron cofactor, enhancing its retention at the active site, a critical factor for enzyme stability. The biotechnological potential of NOV1 was demonstrated using whole cell reactions: cells producing the variant enzyme efficiently converted >99% of 100 mM isoeugenol to vanillin within 24 h. Furthermore, we set up a coupled reaction in which an eugenol oxidase initially converts lignin-derived 4-*n*-propyl guaiacol (4PG) into isoeugenol, followed NOV1 S283F conversion to vanillin. This approach led to a 90% vanillin yield starting from commercial 4PG, and a substantial 66% yield using 4PG from natural lignin oil [2].

To expand our knowledge of NOV1, we identified 20 distal hotspots at >9 Å from the active site using Zymspot software, which predicts advantageous distant mutations, facilitating protein engineering. By combining the resulting most active variants, we generated a double- (12G2) and a triple-mutation (1D2) variants that showed ~10-fold increase in activity and up to 40-fold higher half-life compared to the wild-type [3]. Analysis of the NOV1 crystal structure shows that these mutations, which are placed in a second shell, hypothetically resulted in an enhanced cofactor stabilization and improved kinetic stability of both variants through conformational rearrangement near the active site. These variants show higher immobilization efficiency (>90%) than the wild-type (~60%) in affinity metal resins and performed significantly better in bioconversions where 50 mM of isoeugenol was added stepwise in 24-h cycles.

Overall, our studies highlight the importance of computational tools in elucidating and engineering enzymatic properties. Additionally, we presented two valuable models of isoeugenol/vanillin conversion (whole cell and immobilized enzymes) which, when further developed and optimized at a larger scale, hold promise for economically attractive natural vanillin production.

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DIRECT ELECTRON TRANSFER OF FUNGAL PYRROLOQUINOLINE QUINONE-DEPENDENT PYRANOSE DEHYDROGENASE AND ITS APPLICATION IN A BIOSENSOR

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Redox enzyme catalysis coupled with electrode reaction is termed bioelectrocatalysis and has evolved into a pivotal technology applicable to bioelectrochemical devices, such as biosensors, biofuel cells, and bioreactors. Direct electron transfer (DET)-enabled oxidoreductase has been utilized to develop mediator-free bioelectronic devices. However, most enzymes present a challenge for DET because their catalytic centers are buried within the protein structure and covered with an electrically insulating protein shell. Thus, enzymes that exhibit DET activity remain limited, and the enzymes should be suitably arranged on electrode surfaces.

Here, the fungal PQQ-dependent pyranose dehydrogenase from the basidiomycete *Coprinopsis cinerea* (*Cc*PDH) [1] has been used which has superior DET ability. The full-length *Cc*PDH consists of the catalytic PQQ domain, an N-terminal cytochrome *b* domain, and a C-terminal cellulose-binding domain. The cytochrome domain and the PQQ domain have been classified into the Auxiliary Activities family 8 and 12, respectively, in the Carbohydrate-Active EnZymes database (CAZy). The cellulose-binding domain falls into Carbohydrate-binding module family 1 in CAZy. The oxidation of substrate takes place in the catalytic domain, followed by inter-domain electron transfer (IET) from the reduced PQQ cofactor to the heme *b* in the cytochrome domain.

Notably, both the PQQ domain and the cytochrome domain can perform DET in *Cc*PDH. In the isolated PQQ domain, which lacks the cytochrome domain and the cellulose binding domain in the full-length protein, a high limiting catalytic current of 1.6 mA/cm² was achieved under optimized experimental conditions when the gold nanoparticles-modified electrode was used with a self-assembled monolayer (SAM) of 2-mercaptoethanol [2]. The enzyme catalyzes the oxidation of $^1C_4$ chair monosaccharides such as D-glucosone (2-keto-D-glucose) and L-fucose (6-deoxy-L-galactose). Due to the importance of the L-fucose concentration in urine as a tumor marker, we reported an amperometric L-fucose biosensor based on DET of *Cc*PDH. The sensor allows accurate detection of L-fucose at low potentials where electrochemical oxidation of urinary interferents such as ascorbic acid does not occur [3].

DET is very sensitive to the distance between a protein redox site and the electrode surface. We have been studied the direct bioelectrocatalysis of the full-length enzyme by regulating the distance between the enzyme and electrode with various alkyl chains of self-assembled monolayers (SAMs). Above about 15 Å from PQQ in the active site to the electrode surface, direct bioelectrocatalysis occurred *via* the cytochrome domain by interdomain electron transfer between PQQ to heme, with no catalytic currents obtained by the isolated PQQ domain [4]. The k_{cat} of direct bioelectrocatalysis of full-length CcPDH by the cytochrome domain was maintained at the same level as in the solution assay, suggesting that IET is the rate-limiting step in direct bioelectrocatalysis.

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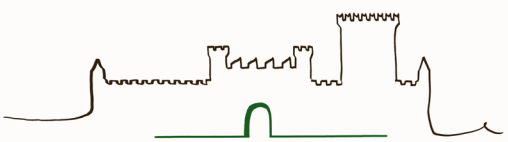

OxiZymes in Lublin

P1. NEW NAD-INDEPENDENT D-LACTATE DEHYDROGENASE FROM GUT MICROBIOME, THROUGH SHOTGUN METAGENOMIC MINING

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Lignocellulosic biomass (LCB) is a sustainable alternative feedstock to produce biochemicals, biomaterials, bioenergy, and biofuels. However, its recalcitrance and heterogeneous structure and composition poses diverse challenges for economical valorisation. Auxiliary Activity (AA) enzymes from the Carbohydrate Active Enzyme database (CAZy) through oxidation of carbohydrates and alcohols, converting them into their corresponding aldehydes or ketones, radical generation and production of hydrogen peroxide enable them to degrade recalcitrant substrates.

In this work, we have explored the *Porcellio dilatatus* gut microbiome as source of new bacterial enzymes to help breakdown lignocellulosic derived materials [1]. Around 20 *P. dilatatus* organisms were collected from two natural LCB-rich environments. Genomic DNA was extracted from their guts and underwent shotgun metagenome sequencing analysis, resulting in 63,409 protein sequences. Using the dbCAN2 software, 1,094 sequences were annotated as CAZymes. The sequences annotated as AA enzymes were analyzed *in silico*, using different bioinformatic tools based on sequence similarity (Blast, Protoparam) and machine learning (CLEAN software, Alphafold). Based on the analysis results, a *Porcellio dilatatus* gut putative D-lactate dehydrogenase (PdG-LDH), belonging to the family AA3, was cloned and heterologous expressed in *Escherichia coli.* the PdG-LDH was kinetically and biochemically characterized. It is a NAD-independent LDH, FAD containing dimeric enzyme with a molecular mass of around 50 kDa. The enzyme exhibited a higher activity (kcat) as a lactate dehydrogenase compared to a lactate oxidase: around 30- to 200-fold higher rates were measured when 1,4-benzoquinone (1,4-BQ) and 2,6-dichlorophenolindophenol (2,6-DCIP) were used as electron acceptor, as compared to oxygen. D-lactate is by far the favorite electron donor: the catalytic efficiency (k_{cat}/K_m) is 3-order of magnitude higher for D-lactate as compared to L-lactate (using1,4-BQ as electron acceptor), at 25ºC, pH 7. Higher efficiency for D-lactate is mostly attributed to the 200-fold lower K_m (0.52 ± 0.06 mM) as compared to that for L-lactate (147 ± 14 mM). The enzyme exhibits a Topt of 55ºC and is thermostable with a Tm of 79ºC. Work is in progress to fully characterize its structure details and give mechanistic insights. NAD-independent LDH enzymes are attractive in the field of biosensors for lactate detection in biological samples [2] and biocatalysis to produce fine chemicals or pharmaceutical intermediates using pyruvate.

These results reinforced the powerful potential of shotgun metagenome sequencing in combination with artificial intelligence (AI)-derived tools in discovering new enzymes.

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P2. ACTIVITY AND SUBSTRATE SPECIFICITY OF BACTERIAL LYTIC POLYSACCHARIDE MONOOXYGENASES (LPMOS) INVESTIGATED THROUGH ATR-FTIR SPECTROSCOPY AND AMPEROMETRY

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Copper enzymes known as lytic polysaccharide monooxygenases (LPMOs) can breakdown resistant substrates in environmentally friendly ways. The catalytic peroxygenase mechanism has yet to be fully characterized, but their catalytic peroxygenase mechanism has yet to be fully understood. This is also because the analysis of reaction kinetics must deal with multiple variables, including the nature of the substrates, the undesirable side reactions, and the low protein stability in the presence of H_2O_2 as co-substrate. In this study, the peroxygenase activity of three bacterial LPMOs belonging to the AA10 family, namely *Pp*AA10, *Sc*AA10B and *Sc*AA10C was studied through two novel methods, an ATR-FTIR assay for qualitative analysis and an amperometric assay for kinetic characterization, that allowed the determination of the turnover number (TN), the total turnover number (TTN) and the residual activity on different substrates to be determined, providing a valuable insight into their substrate specificity and stability. The results show that bacterial LPMOs have fast peroxygenase reactions, suggesting that they are real peroxygenases rather than monooxygenases. Furthermore, the presence of the carbohydrate-binding module (CBM) is demonstrated to be required for enabling fast and stable LPMO reactions, especially in the presence of high concentrations of H_2O_2. Although the initial rates were lower than those for fungal LPMOs, the enzyme stability increases over time on more crystalline substrates and in the presence of the carbohydrate-binding module (CBM), as in *Sc*AA10C, providing values comparable to fungal LPMOs. Moreover, the presence of CBM allowed LPMO to consume H_2O_2 more efficiently, resulting in higher enzymatic activity and oxidation resistance to hydrogen peroxide.

P3. SELECTIVE SYNTHESIS OF EPOXY LIPID MEDIATORS BY UNSPECIFIC PEROXYGENASES

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Lipid mediators are important human metabolites that modulate renal function, angiogenesis, vascular dilatation and inflammation, regulate monocyte aggregation and are involved in cardiovascular and metabolic diseases [1,2]. The metabolic route for generation of these compounds englobes the oxidation and oxygenation of polyunsaturated fatty acids (PUFAs) by cytochrome P450 enzymes (CYP), resulting in epoxy derivates (EpFAs). Further research on the topic has been limited by the unstable nature of EpFAs in cell systems and the small amount of cell-produced derivates. The application of synthesis techniques which can overcome this limitation is presented in this work. Fungal unspecific peroxygenases (UPOs), a group of enzymes that possesses CYP-like catalytic activity, are able to produce EpFAs from PUFAs. in this work, the enzymes TanUPO, rPabUPO-II and rPabUPO-I were applied to a buffered mixture with arachidonic acid (AA), eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), respectively. The isolated products were 14,15-epoxy-5,8,11-eicosatrienoic acid (14,15-EET); 17,18-epoxieicosa-5,8,11,14-tetraenoic acid (17,18-EEQ) and 19,20-epoxi-4,7,10,13,16-docosapentaenoic acid (19,20-EpDPA), from the respective PUFAs. The obtained molecules were verified by ^{1}H-NMR and ^{13}C-NMR. Our results demonstrate the capacity of UPOs to synthesize lipid mediators for further research, diagnostic and medical application.

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P4. BIOGRAFTING OF INDUSTRIAL POLYMERS TO IMPROVE CARDBOARD PROPERTIES

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Plastic is the main material in the packaging industry reaching a high production of them ($3.80x10^8$ t per year), but its origin from fossil resources and negligible biodegradability is causing environmental issues. In this context, paper and cardboard are gaining a lot of interest, but some of their properties reduce its potential such as their low compatibility with water, low antimicrobial resistance, low strength and are impossible to sterilize and close hermetically. Some of those disadvantages can be fixed by employing coatings or modifying the structure with a wide variety of compounds to improve its hydrophobic behavior or increase the antimicrobial properties of cardboard. Plastic, aluminum and starch can be used for this purpose [1]. Starch highlights as a promising coating due to its abundance, its renewable source biodegradability and low cost. However, some disadvantages exist like low water stability, high moisture sensitivity, poor barrier to humidity and low antimicrobial resistance [2]. Some of those drawbacks can be solved by integrating hydrophobic compounds in the starch formulation, like phenolic compounds, to increase water stability, develop antioxidant properties and enhance antimicrobial properties. a novel strategy to modify the starch is the biografting of phenolic compounds employing laccases that create new bonds between the starch and the oxidized phenolics. This binding permits the modification of starch without the possibility to be released to the possible packed materials [3]. The phenolic compounds can come from different sources like winemaking industry residues. Those residues are one of the most bioactive and plentiful, specially in Spain. Grape pomace (GP) and vine pruning (VP) stand out as the most promising one with high phenolic content and high production [4]. So those phenolics will be tried to biograft onto different polymers to improve their properties.

Laccase from *Myceliophthora thermophila* (Novozym 51003®, Novozymes, Denmark) will be employed. Ferulic acid will be tested as phenolic acid model compound along with different phenolics liquors from the pretreatment of winemaking industry residues. The oxidation of this compounds to produce free radicals will be followed by radical coupling that will enable the covalent binding of phenolics. Those modifications will be studied by different methods like UV-vis spectrum, HPLC coupled with a diode-array detector (for phenolic determination), determination of total phenolic compounds (Folin-Ciocalteu method), determination of antioxidant capacity (DPPH and FRAP method) and determination of antimicrobial properties, along with different spectrometry techniques.

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P5. EVALUATION OF OXALATE DEGRADING FUNGI AS A POTENTIAL SOURCE OF HYDROGEN PEROXIDE FOR REACTION CATALYSED BY UPO

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Fungi, as well-known producers of oxalic acid, also possess an active and efficient system for regulating its concentration in the surrounding environment. This system involves the action of enzymes, from the class of liases – oxalate decarboxylase (ODC, EC 4.1.1.2) and class of oxidoreductases – oxalate oxidase (OXO, EC 1.2.3.4) [1]. The efficient degradation of exogenous oxalate by action of OXO can provide a source of hydrogen peroxide required for the catalytic cycle of fungal peroxidases and peroxygenases. The generation of hydrogen peroxide by OXO is a simple and efficient system that uses only one enzyme and generates volatile CO_2 as a by-product. Unspecific fungal peroxygenase (UPO, EC 1.11.2.1) is able to incorporate one oxygen atom from the peroxide into the product, providing an excellent tool in the oxyfunctionalization biocatalysis. There are now also strong indications of the widespread occurrence of UPO throughout the fungal kingdom [2].

The data presented allowed us to verify the mode of oxalic acid decomposition in cultures of various Basidiomycetous fungi in order to determine whether the oxidative oxalate decomposition pathway could be a common and efficient source of hydrogen peroxide. The fungi analyzed could potentially degrade oxalic acid via the oxidative pathway, leading to the production of hydrogen peroxide. We have found different patterns of hydrogen peroxide generation by the fungi studied during oxalate degradation.

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P6. BIOCHEMICAL CHARACTERISATION OF A RECOMBINANT LACCASE FROM *HALALKALIBACTERIUM HALODURANS* C-125 AND ITS APPLICATION IN THE BIOTRANSFORMATION OF ORGANIC COMPOUNDS

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Halalkalibacterium halodurans bacteria live in alkaline and hypersaline conditions [1], which makes the species interesting host-producing enzymes. *H. halodurans* C-125 with a whole-sequenced genome has been shown to produce lignocellulosic enzymes [2] and some of them have been proved to be active at high temperature and pH values [3,4].

In the present study, a gene encoding a putative laccase from *H. halodurans* C-125 was cloned within *E. coli* BL21 (DE3), resulting in a recombinant version of the enzyme. The recombinant laccase was expressed as a protein lacking the native signal peptide sequence for the intracellular expression of the protein and designated as rLac-HhC125. The rLac-HhC125 was partially purified, biochemically characterised, and applied for the transformation of organic compounds belonging to methoxy-, hydroxy-, and amino-derivatives as precursors for dye synthesis. The rLac-HhC125 showed a capability of efficient transformation of organic compounds into coloured molecules.

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P7. FUNGAL CO-CULTURES AS A MODERN BIOTECHNOLOGICAL TOOL FOR MODULATING OXIDASES ACTIVITY

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The co-culture systems have been successfully used to study cell-cell interactions. Studies indicate that interactions between different species within the same co-culture can influence inhibiting or inducing the reactivity of various biochemical enzymatic end non-enzymatic parameters [1]. The representatives of white rot fungus belonging to the *Basidiomycota* are characterized by their ability to synthesize intracellular and extracellular oxidoreductive or hydrolytic enzymes e.g. laccase, protease, or peroxidase, with high stability and activity [2]. In the presented work for the first time, the following three types of proprietary fungal co-cultures were conducted: (1) *Cerrena unicolor* + *Fomitopsis betulina*, (2) *Cerrena unicolor* + *Abortiporus biennis*, and (3) *Cerrena unicolor* + *Trametes suaveolens*. All selected component organisms demonstrated a significant biotechnological potential [3-6]. The purpose of this study was to comprehensively analyze the impact of fungal co-culture on the metabolic activity of experimental systems with a particular reference to laccase and *β*-glucosidase, which belong to the oxidase enzymes group. Moreover, all co-cultures were conducted under shaking and stationary conditions. In order to obtain a detailed review of the changes regarding metabolic activity within co-cultures, respective fungal monocultures included in co-culture were used as controls. Laccase was examined applying two methods, (a) spectrophotometrically with syringaldazine as a reaction substrate and (b) *via* electrophoretic detection under native conditions. The highest stimulation of laccase production was noticed in co-culture of *C. unicolor* + *T. suaveolens* [5.3 ± 0.68 nkat/μg], conducted under shaking conditions, within the 5th testing day. Surprisingly, in co-culture containing the same species but carrired out under stationary conditions, laccase's activity was markedly inhibited. *β*-glucosidase activity was marked using an electrophoretic detection technique. The study reveals that stimulation or inhibition of *β*-glucosidase activity depends on the co-culture variant as well as the breeding conditions.The significant changes were observed also in morphological characteristics of the mycelium of selected white wood rot fungal species growing together on solid media.

In conclusion, the results obtained in this work present a new potential use of fungal co-cultures as a modulator of fungal oxidases production and catalytic activity, and might be considered as a highly-efficient tool in biotechnology, medicine, and industrial applications.

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P8. BIOCATALYTIC SYNTHESES OF ANTIPLATELET METABOLITES OF CLOPIDOGREL AND PRASUGREL USING FUNGAL PEROXYGENASES

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Antithrombotic thienopyridines, such as clopidogrel and prasugrel, are prodrugs that undergo a metabolic two-step bioactivation for their pharmacological efficacy. In the first step, a thiolactone is formed, which is then converted by cytochrome P450-dependent oxidation *via* sulfenic acids to the active thiol metabolites [1]. These metabolites are the active compounds that inhibit the platelet P2Y12 receptor and hereby prevent atherothrombotic events. So far, described biocatalytic and chemical synthesis approaches to obtain active thienopyridine metabolites are rather complex and suffer from low yields [2]. In the present study, several unspecific peroxygenases (UPOs, EC 1.11.2.1) known to efficiently mimic P450 reactions *in vitro* – but requiring only hydroperoxide as oxidant – were tested for biocatalytic one-pot syntheses. The preparation of the active metabolite of clopidogrel was successful *via* a two-step oxidation using a UPO from the agaric fungus *Marasmius rotula* (*Mro*UPO) with an overall yield of 25%. In the case of prasugrel, a cascade of porcine liver esterase (PLE) and *Mro*UPO was applied, resulting in a yield of 44%. The two metabolites were isolated with high purity and their structures confirmed by MS and MS2 spectrometry as well as NMR spectroscopy. The findings broaden the scope of UPO applications and demonstrate that they can be effectively used for the selective synthesis of metabolites and late state diversification of organic molecules, circumventing complex multi-stage chemical syntheses and providing sufficient material for structural elucidation, reference material, or cellular assays.

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P9. DEVELOPMENT AND SCREENING OF UNSPECIFIC PEROXYGENASE VARIANTS FOR IMPROVED AROMATIC HYDROXYLATION OF SUBSTITUTED BENZENES

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Unspecific peroxygenases (UPOs) are heme-thiolate enzymes secreted by fungi, that combine the activity of peroxidases and peroxygenases. By using hydrogen peroxide, these enzymes oxidize a variety of organic compounds without the need for additional cofactors [1]. Within the last years, the number of discovered UPOs and evolved UPO variants for application as biocatalysts strongly increased [1,2]. However, heterologous expression of these enzymes at high yields is still a challenge.

We identified a new short UPO from *Aspergillus brasiliensis* (*Abr*UPO), which was heterologously expressed in the methylotrophic yeast *Pichia pastoris* (reclassified as *Komagataella phaffii*) at up to 750 mg/L culture medium [3]. In contrast to other UPOs, *Abr*UPO catalyzes the oxidation of substituted benzenes at both, the aromatic and benzylic positions. The preference of *Abr*UPO for aromatic or benzylic hydroxylation depends on the chemical properties and the alkyl chain length of the substituted benzenes.

We aimed to improve aromatic hydroxylation by introducing mutations in the active site and in the substrate access channel of *Abr*UPO. In order to screen the constructed *P. pastoris* transformants for the most productive clones with little effort, within short time, and without automated screening devices, we developed an agar plate-based activity assay [4]. Thereby, cell growth and protein expression were conducted on agar plates supplemented with methanol and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) for up to 3 days. An additional top agar layer supplemented with ABTS and peroxide was then added, leading to the formation of green zones around UPO-secreting *P. pastoris* transformants within 15 minutes. The assay was validated with *Abr*UPO and evolved PaDa-I from *Agrocybe aegerita*. The assay was successfully applied to screen several constructed single, double and triple variants of *Abr*UPO for the most productive *P. pastoris* transformants to be produced at the 200 ml-scale in shaking flasks. Investigation of the activity and chemoselectivity of the constructed *Abr*UPO variants has revealed, that both were influenced by the introduced mutations.

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P10. DIRECT OXIDATION OF THE DOUBLE BOND WITH H_2O_2 BY THE MYCELIUM OF *CLADOSPORIUM CLADOSPORIOIDES* 01

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Epoxidized olefins are the most appropriate intermediates for industrial production because of the high reactivity of epoxy bond. They are used to synthesize polymers and compounds with various biological activities [1]. Obtaining epoxides via chemical synthesis negatively affects the environment. Therefore, an increasing number of studies have focused on the use of biotechnological methods for the production of epoxy compounds [2]. Catalysts of biological origin enable the reaction to be carried out with high efficiency at moderate temperatures and atmospheric pressure.

The aim of this study was to determine the activity of direct oxidation of the limonene double bond in the presence of H_2O_2 using freeze-dried mycelia of *Cladosporium cladosporioides* 01.

In the first stage of the study, fatty acids contained in the mycelia were washed out using ethyl acetate (16 h) and hexane (16 h). Washing out the fatty acids was necessary to eliminate the possibility of chemoenzymatic epoxidation of limonene, in which the oxidizing agent would be peroxyacids. The biocatalyst prepared in this manner was used to oxidise limonene (58 µL) in toluene with 30% hydrogen peroxide (164 µL). The reaction was performed without stirring for 3 h at 55 °C. Subsequently, 6.5% oxidation of limonene to limonene 1,2-epoxide (5.7%) and limonene 8-9-epoxide (0.8%) was obtained. This is a high activity for direct oxidation of the double bond compared to the oxidation of but-2-enal in acetonitrile by lipase B from *Candida antarctica*, where 44% oxidation was achieved within 17.5 days [3]. Further research on the oxidation mechanism and the influence of the reaction conditions on its efficiency is necessary.

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P11. DISCOVERY OF ALKALINE LACCASES FROM BASIDIOMYCETE FUNGI THROUGH MACHINE LEARNING BASED APPROACH

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Laccases can oxidize a broad spectrum of substrates, offering promising applications in extensive various sectors. However, laccase discovery and optimization of laccases with a desirable pH optimum remains a challenge due to the labor-intensive and time-consuming nature of the traditional laboratory methods. This study presents a machine learning (ML) integrated approach for predicting pH optima of basidiomycete fungal laccases, utilizing a small, curated dataset against a vast metagenomic data. Comparative computational analyses unveiled structural and pH-dependent solubility differences between acidic and neutral-alkaline laccases, helping us to understand the molecular bases of enzyme pH optimum. The pH profiling of the two selected ML-predicted alkaline laccase candidates from the basidiomycete fungus *Lepista nuda* further validated our computational approach, showing the accuracy of this comprehensive method. This study uncovers the efficacy of ML in the prediction of enzyme properties from minimal datasets, and marks a significant step towards harnessing computational tools for the systematic screening of enzymes for industrial and environmental biotechnology applications.

P12. CHARACTERIZATION OF A PYRANOSE OXIDASE/C-GLYCOSIDE OXIDASE FROM *MICROBACTERIUM* SP. 3H14, BELONGING TO THE UNEXPLORED CLADE II OF POX/CGOX

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Pyranose oxidase (POx; EC 1.1.3.10) is an FAD-dependent oxidoreductase and belongs to the glucose-methanol-choline (GMC) superfamily of oxidoreductases. POxs oxidize various aldopyranoses at the C2-position in the presence of molecular oxygen, producing 2-ketoaldoses and hydrogen peroxide [1]. It was recently shown that POxs and *C*-glycoside oxidases (CGOx), which preferentially oxidize the glucose moiety of various *C*- and *O*-glycosides, share the same sequence space. Phylogenetic analysis of actinobacterial sequences belonging to this shared sequence space revealed that it can be divided into four clades. However, no extant POx has been characterized from one of these clades so far [2]. The aim of this project was to study a POx from *Microbacterium* sp. 3H14 (*M*POx), belonging to this unexplored clade II.

Previously characterized, bacterial POxs/CGOxs show varying activity with different monosaccharides and glycosides [2]. Therefore, substrate screening with *M*POx was performed using different monosaccharides and glycosides as possible electron donors. In addition, different electron acceptors were tested as well, and we used selected substrates to determine kinetic parameters. Stability studies were conducted, and the pH optimum, pH stability, thermostability and half-life times were determined. Moreover, as monomeric and dimeric bacterial POxs/CGOxs were reported [3, 4], the oligomeric state of the protein was determined using size exclusion chromatography. Finally, RoseTTAFold (https://robetta.bakerlab.org/) was used to predict the structure of *M*POx, which was further compared to known structures of closely related enzymes.

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P13. THE INFLUENCE OF LOW MOLECULAR WEIGHT COMPOUNDS FROM CULTURES OF WOOD DEGRADING FUNGI ON THE ACTIVITY OF OXIDASES ENZYMES

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Wood decaying fungi belonging to the Basidiomycota are recognized as a one of the most interesting groups of biotechnology useful organisms. A wide range of bioactive substances isolated from fungi can generally be classified into two groups: high molecular weight compounds, which include polysaccharides and proteins and low molecular weight compounds, such as indoles, terpenoids, or phenols [1].

In the presented work the influence of low molecular weight secondary metabolites (ex-LMS) subfractions from *Armillaria mellea* and two strains of *Trametes versicolor* culture fluids on the activity of selected enzymes with biotechnological potential (fungal laccase, tyrosinase and acetylocholinoesterase) was determined. Three different low molecular weight fractions with a mass below 10 kDa obtained from the post-culture fungal liquids were examined for their possible modification of chosen enzymes activities. In order to determine the impact of the tested low molecular weight fraction on the activity and stability of enzymes, a 7-day ex-LMS incubation was carried out with three different concentrations of ex-LMS fractions (5 mg/ml, 10 mg/ml and 15 mg/ml, in ratio 1:1 with selected enzymes solution), and then the enzymatic activities were checked. LAC activity was measured following oxidation of 0.025 mM of syringaldazine (4-hydroxy 3,5-dimetoxybenzaldehyde) in 50 mM buffer at pH 5 [2]. Tyrosinase inhibition assays were performed with L-DOPA as substrate. The reaction mixture contained 150 µL of phosphate buffer (100 mM, pH 6.8), 30 µL of incubation mixture and 20 µL of 2,5 mM L-DOPA. After the addition of L-DOPA the reaction was immediately monitored at 475 nm for dopachrome formation in the reaction mixture [3]. The enzymatic assay for acetylcholinesterase (AChE) activity was performed utilizing spectrophotometric method. Briefly, 130 µL of 0.1 M sodium phosphate buffer (pH 8), 30 µL of incubation mixture were added in a 96-well microplate. After an incubation period of 15 mn at 25^{O}C, the reaction was then initiated by the addition of 20 µL of DTNB and 20 µL of substrate acetylthiocholine iodide [4]. The formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction was monitored spectrophotometrically at 412 nm using a 96-well microplate reader. Determination of enzymes activities in the presence of modifying fungal preparations in the case of laccase basically showed a significant decrease in enzyme activity. Based on the analyzes of the level of acetylcholinesterase and tyrosinase activity on three different days of incubation of the enzyme with ex-LMS, it follows that the higher the concentration of the tested fraction of secondary metabolites, the greater the decrease in enzyme activity.

The obtained results indicate that the introduction of a fungal ex-LMS fractions into the reaction mixture allows for a clear modification of the activity of the tested enzymes (fungal laccase, tyrosinase and acetylocholinoesterase), which seems significant from the practical application point of view.

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P14. DEVELOPMENT OF ARTIFICIAL ENZYMES FOR THE PHOTOCONVERSION OF WASTE TO HYDROGEN

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The development of new materials for the conversion of waste into useful products is important in the context of green energy transition. As a promising route for sustainable utilization of waste, we propose enzyme biomimicry combined with light-induced reaction. For that purpose, the stable-protein-1 (SP1) will be used as a highly robust scaffold for the construction of biocatalysts with enzyme-like activity. Through a combined theoretical and experimental approach we plan to develop a library of artificial enzymes, in particular: a) peroxidase artificial enzyme for degradation of waste into value-added products and b) flavin-based fatty acid photodecarboxylase (FAP) artificial enzyme for the conversion of fatty acids into fuels.

P15. EFFECT OF POLYMERS OBTAINED FROM *TRICHODERMA KONINGIOPSIS* CULTURES ON ANTIOXIDANT ENZYME ACTIVITY IN WHEAT TISSUES

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Species belonging to the genus *Trichoderma* directly and indirectly influence plant growth and development and are thus used in biopreparations [1]. However, the use of living microorganisms is fraught with problems in adaptation to variable soil environments. Therefore, it seems interesting to use the metabolites they synthesise as elicitors, that is, substances that directly influence the enzymatic pathways of plant resistance [2]. Fungal polymers seem to be an interesting group of compounds [3]. These compounds are recognised by plant receptors and trigger a number of cellular reactions. One of the most important enzymes involved in resistance pathways is oxidoreductase (catalases, peroxidases, dismutases, and reductases) [4].

The aim of this study was to obtain polymers (EPS and WPS) from *Trichoderma koningiopsis* Tk3Ag0 cultures and to determine their structure and elicitor properties. EPS were obtained from a medium containing 3% sucrose and 0.75% peptone. Cell wall polymers were extracted from the biomass obtained using a step-by-step method: cold water-soluble (WPSZ), hot water-soluble (WPSC), and alkali soluble (WPSNaOH) fractions. The obtained PS fractions were then subjected to purification (deproteinisation and dialysis) and structural analysis (GC-MS, fraction size, and bond types). To determine the elicitor capacity, the seeds of winter wheat cv. Arcadia were germinated in the presence of 0.05% and 0.1% PS suspensions. The seedlings were grown for 10 days, after which the stems were separated from the roots and intracellular proteins were extracted.

The activities (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) were determined in the extracts obtained. The greatest increase in enzyme activity was observed after the application of the EPS fractions at 0.05% and 0.1% and the WPSNaOH fraction at 0.05%, where an almost 2-3-fold increase in enzyme activity in tissues was observed. The ability to stimulate plant resistance at the oxidoreductase level demonstrates the influence of the obtained PS on antioxidant pathways, which are among the first lines of plant defence against the action of phytopathogens. Thus, the obtained PS can be used to formulate biopreparation.

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P16. ALTERING THE PROPERTIES OF FUNGAL AND BACTERIAL LACCASES BY CHEMICAL MODIFICATIONS OF PROTEINS

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Due to their catalytic performance, laccases (EC 1.10.3.2) constitute one of the most promising groups of enzymes for potential applications in modern biotechnology [1,2]. In this study, we aimed to comparatively characterize the structure of *Sinorhizobium meliloti* [3] and *Cerrena unicolor* [4] laccase in order to improve and elucidate the efficacy of further chemical modification. The analysis showed that bacterial laccase was characterized by a more hydrophobic solvent-accessible surface area than the fungal enzyme. Next, we chemically modified the enzyme free amino acid residues *via* glycosylation using a polymer compound. The stability of covalently modified laccases over a wide pH and temperature ranges and in the presence of inhibitors was investigated. Protein modifications significantly boosted enzyme activity. The alteration in the pH optima of the laccases *via* chemical modification was hard to achieve, but improved stability at neutral pH was observed. The basidiomycetous laccase proved to be more resistant to elevated temperature than the bacterial one. It appears that this simple modification methodology may offer a helpful strategy for imparting desirable stability and catalytic activity to laccases.

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P17. DESIGN OF BIOCATALYTIC OXIDATIVE REACTIONS WITH DEEP-EUTECTIC SOLVENTS

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Biocatalysis represents a promising pathway for transforming bio-renewable starting materials into valuable industrial products. While water remains the predominant solvent in many biocatalytic processes, its high polarity often clashes with the hydrophobic nature of many target substrates, limiting substrate solubility and constraining the metrics of process intensification. Addressing this challenge necessitates a concerted sci-tech approach to integrate sustainable non-aqueous media into biocatalysis. In recent years, deep eutectic solvents (DESs) have emerged as promising alternatives to conventional solvents like organic solvents and ionic liquids (ILs) in biocatalytic applications. Offering advantages such as cost-effectiveness, biodegradability, biocompatibility, and sustainability, DESs have garnered significant attention. With this aim DECADES (DEsign of CAtalytic processes with Deep-Eutectic-Solvents) was conceived as a multinational doctoral network project funded by the European Union, dedicated to exploring the potential of DESs in biocatalysis. Our mission is to enhance the sustainability of biotechnological processes by leveraging DESs as highly advantageous solvents. To achieve this goal, DECADES has outlined the following objectives:

a) tailoring biocatalytic activity and stability for DES in representative reactions as aerobic oxidative modification of lignin-derived phenolics,

b) investigating the effects of DES on enzymes and bio-/chemo-catalytic systems, and developing optimization strategies,

c) demonstrating the feasibility of DES technology by implementing production routes for target products such as highly functionalized lignin-based phenolics.

To fulfill these objectives we are implementing a research agenda consisting in:

a) study of development of kinetic modelling of the laccase-catalyzed oxidation of phenolics in DESs,

b) development of solid-supported laccase biocatalysts with high activity-stability in water-DESs mixtures,

c) study of the relationship of oxygen uptake rate in laccase-catalyzed oxidation of phenolics and oxygen transfer rate in DESs.

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P18. TWO NOVEL UNSPECIFIC PEROXYGENASES IDENTIFIED BY A SIMPLE AGAR PLATE BASED SCREENING

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Fungal unspecific peroxygenases (UPO, EC 1.11.2.1) enable the synthesis of active pharmaceutical metabolites and consequently represent a valuable tool in synthetic chemistry. So far, the identification of novel, recombinantly expressible UPOs has been slowed down by time-consuming methods and expensive equipment. In this study, a simple high-throughput agar plate-based screening method is described to detect the presence of heterologously expressed UPOs in the yeast *Saccharomyces cerevisiae*. Using this method, two short UPOs from the filamentous fungi *Dendrothele bispora* (*Dbi*UPO) and *Aspergillus niger* (*Ani*UPO) were successfully identified in a small gene library containing putative UPO genes. Investigation of their catalytic properties showed their potential for the formation of human drug metabolites, e.g. lipid mediators from polyunsaturated fatty acids or the active metabolites of the prodrug clopidogrel, with varying efficiency and product specificity.

P19. LACCASE-MEDIATED SYNTHESIS AND PROPERTIES OF NOVEL BIOACTIVE DYE

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Biocatalysis processes based on oxidoreductases, such as laccase, are important for discovering new organic compounds with broad structures and potential applications [1-3]. They include bioactive compounds, which can be obtained through modification of well-known bioactive agents but also through de novo oxidation of organic substrates having hydroxyl and/or amino groups.

In the present work, 5-aminosalicylic acid (5-ASA) was examined as a potential substrate for fungal laccase due to the presence of hydroxy and amine groups, the main substituents involved in the oxidation and coupling reaction mediated by fungal laccase [4]. Substrate was transformed by fungal laccase from *Cerrena unicolor* strain into a novel red-brown coloured compound [5]. Crude transformation product was analysed to assess its bioactivity, toxicity and application for wool dyeing. the biotransformation process was optimised especially in terms of the pH value, buffer and co-solvent concentration, and laccase activity.

The data obtained clearly indicated that a low concentration of the reaction buffer in the pH range from 5 to 6 and in the presence of 10% acetonitrile increased the rate of substrate oxidation and the amount of the product formed. The red-brown compound obtained via laccase-mediated oxidation of 5-aminosalicylic acid showed antioxidant properties and unique antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* strains with the MIC value of 0.125 mg/mL detected for the purest dye. Obtained compound was reported to have good wool fibre dyeing properties and no irritant effect after patch tests on a selected group with increased skin sensitivity, which confirms the usability of this product as a bioactive dye.

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P20. 8-TRANS-HYDROXYCALAMENENE ATROPISOMERS: CHEMO-ENZYMATIC SYNTHESIS AND BIOLOGICAL EVALUATION

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Sesquiterpenes display several biological activities, exemplified by representative compounds like calamenene, which exhibits antimalarial properties [1]. Since introducing a hydroxyl group in the C-7 position of the calamenene skeleton has shown promising leishmanicidial activity [2] and there are no similar biological evaluations concerning 8-hydroxycalamenene (8-HC), this molecule has caught our attention.

8-*trans*-HC, with fish toxicity and antibacterial activity in essential oil mixtures [3], was never examined as isolated molecule, and no biological data is available regarding its literature-reported C-C dimers [4]. Thus, the two enantiomers of 8-*trans*-HC were chemically prepared through a stereoselective synthetic protocol involving a homologation-benzannulation sequence [5] starting from naturally occurring (-) and (+)-menthol.

Their separate oxidative C-C couplings, catalyzed by *Trametes versicolor* laccase, led to the isolation of the target dimers as stereoenriched mixtures of stable and separable diastereoisomeric atropisomers.

The subsequent spectrophotometric characterization, combined with a DFT-computational approach, confirmed their mutual relationships as enantiomers and allowed to assign their specific absolute configurations. Both the 8-*trans*-HC enantiomers exhibited micromolar range activity against drug-resistant strains of *Plasmodium falciparum*, and the more active dimers also showed low cytotoxicity.

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P21. ACTIVITY AND MECHANISMS INVOLVED IN MANGANESE OXIDATION IN DYP-TYPE PEROXIDASES

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The conversion of Mn(II) to diffusible Mn(III) is an essential aspect of the synergistic microbial degradation of lignin observed in nature, a trait commonly associated with basidiomycete manganese peroxidases (MnPs) and versatile peroxidases (VPs), but also reported in bacterial and fungal DyP-type Peroxidases (DyPs). This family is a relatively recently characterized microbial heme-containing enzymes that show attractive properties for biotechnological purposes by oxidizing various substrates, including synthetic dyes, metals, and phenolic and non-phenolic compounds derived from lignin. DyPs can be categorized into three classes according to structure-based sequence alignments: class P (Primitive, formerly class B), class I (Intermediate, formerly class A), and class V (Advanced, formerly classes C and D).

The Mn(II) binding site typically consists of acidic residues proximal to the heme propionate in X-ray crystal structures of fungal MnPs and VPs complex with manganese. In the case of DyPs, the location of the Mn(II) binding site appears to vary across the different classes. In some DyPs (class P), the acidic residues involved in Mn(II) binding are near the substrate entrance cavity, similar to fungal counterparts, suggesting a more direct interaction (electron transfer) between the Mn(II) and the heme [1]. However, in the other classes of DyPs, the acidic residues putatively responsible for Mn(II) binding are situated on the protein surface, distant from the heme, implying a long-range electron transfer (LRET) pathway to the heme pocket, involving neighboring (aromatic) residues [2].

Based on structural sequence alignments and available X-ray structural data complexed with manganese, we have hypothesized on the Mn(II) binding sites in *Bacillus subtilis* DyP (*Bs*DyP) and *Pseudomonas putida* (*Pp*DyP), which belong to class I and class P, respectively [3-5]. In *Pp*DyP, a negative residue patch is located within the substrate cavity close to the heme propionate, which could be involved in Mn(II) catalysis. In contrast, in *Bs*DyP, two clusters of acidic residues were identified distal to the heme pocket, implicating LRET. We are currently using site-directed mutagenesis targeting the hypothetical residues responsible for Mn(II) binding that, coupled with X-ray studies involving analysis of structures complexed with manganese, will elucidate the mechanisms underlying manganese oxidation in DyP-type peroxidases.

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P22. OXIDATION OF ALIPHATIC AND AROMATIC ALCOHOLS BY *CERRENA UNICOLOR* ALCOHOL OXIDASE

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Alcohol oxidase (AOX) EC. 1.1.3.13 is an oxidoreductase obtained most often from methylotrophic yeasts such as *Pichia pastoris* but also Basidomycetous fungi. Recently, this enzyme was isolated and characterized from *Cerrena unicolor.* Alcohol oxidase carries out the process of oxidizing alcohols to the corresponding aldehydes, producing hydrogen peroxide as a reaction by-product. This catalytic ability may be employed to synthesize biotechnologically important aldehydes: benzaldehyde or cinnamaldehyde. In the experiment carried out using high-performance liquid chromatography, it was found that alcohol oxidase oxidizes primary and aromatic alcohols to specific aldehydes with high specificity. The high degree of conversion of primary alcohol into aldehyde was observed when reacting with methanol. The degree of conversion of methanol into formaldehyde was 4.36 ± 0.27. This is obvious because the specific reaction of SCAO alcohol oxidase is the oxidation of methanol, as the simplest primary alcohol. Interestingly, the research showed that the highest percentage of transformation was observed during the reaction of alcohol oxidase with 4-hydroxybenzyl alcohol, as much as 13.97 ± 0.83. Aromatic compounds such as benzyl alcohol and its derivatives are oxidized to the corresponding aldehydes by aryl-alcohol oxidase AAO.

P23. CELLOBIOSE DEHYDROGENASE AND LACCASE AS BIOTECHNOLOGICAL TOOLS IN THE ALDONIC ACIDS SYNTHESIS

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Cellobiose dehydrogenase and laccase are oxidoreductive enzymes with broad biotechnological potential. Cellobiose dehydrogenase (CDH, EC 1.1.99.18) is an extracellular flavoenzyme containing two domains (flavin and heme) linked by a flexible linker. CDH catalyses the oxidation reaction of di- and oligosaccharides linked by β-1,4-glucosidic bonds, such as cellobiose and lactose, to the corresponding lactones, which spontaneously convert to aldonic acids [1]. Laccase (LAC, EC 1.10.3.2), a *p*-diphenol oxidase, is a multi-copper oxidase (MCO) and oxidise organic and inorganic compounds such as mono-, di-, poly-, amino- and methoxyphenols and several aromatic amines [2] while reducing molecular oxygen to water.

An interesting perspective is the use of both biocatalysts in the synthesis of carbohydrate derivatives such as aldonic acids, which are formed by the oxidation of aldoses and have great application potential. The oxidation products of lactose or cellobiose (lactobionic acid or cellobionic acid) have found many applications in the pharmaceutical, food and cosmetic industries due to their antioxidant, antimicrobial, moisturising and chelating properties, as well as prebiotic effects. An important direction of research is the immobilisation of enzymes on suitable matrices used in the synthesis of aldonic acids, which reduce the cost of the biosynthesis process while increasing efficiency and to create multi-enzymatic systems, increasingly used in industrial processes.

The aim of the present research was a comprehensive approach to the use of oxidoreductive enzymes in the synthesis of aldonic acids. The research conducted includes the evaluation of the physicochemical and biological properties of native and immobilised enzymes, the characterisation of carriers and immobilised enzymes in the context of efficient synthesis.

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P24. BIOSORPTION OR BIODEGRADATION – COMPARATIVE STUDY FOR BISPHENOL A CONTAMINANT ELIMINATION

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Bisphenol a (BPA) is a commonly used compound in the production of plastic items; however, it is detrimental to humans and aquatic organisms because of its endocrine activity and is still ineffectively removed from the environment [1]. Among numerous solutions, the most popular is the fungal-based method of BPA degradation with lignin-modifying enzymes applied as purified or secreted indirectly by the active mycelium [2,3]. Second, the rarely studied method is sorption by fungal biomass, yet it is still promising as it is easy-to-use and does not generate harmful byproducts.

The data presented are a comparative study of two fungal-based methods: enzyme degradation and biomass sorption of BPA.

The biological material used in this study was fungi belonging to the *Pleurotus* genus. Laccase was obtained in submerged culture of *P. ostreatus*, subsequently purified using ion-exchange chromatography, and use in native and immobilised form, while homogenates of *P. ostreatus* (cultivated and wild), *P. eryngii, P. citrinopileatus, P. djamor* and *P. pulmonarius* were used as sorbents. Spectrophotometric methods were used for BPA detection in the solution before and after treatment, and the starting concentrations of BPA were 10 mg/L and 100 mg/L for enzyme (native/immobilized) and sorbent removal, respectively. The obtained results were shown as percentage of BPA removal and calculated on m^3 of treated solution, assuming that the BPA concentration was mostly reported in the literature. The results showed that the sorption of BPA and its degradation by native laccase are comparably efficient methods under optimal conditions, whereas immobilised laccase showed much less effectiveness.

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P25. ACTINOBACTERIA - EFFICIENT PRODUCERS OF VALUABLE ENZYMES

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Microorganisms represent a rich source of compounds used in both industry and pharmacy. Essential are Gram-positive bacteria belonging to the Actinobacteria, commonly found in soil, compost, and water. This group of bacteria is well known for its unique ability to adapt to extreme environmental conditions. Substances synthesized by Actinobacteria include enzymes, antibiotics, dyes, fungicides, and insecticides [1].

The synthesis of various enzymes, enables Actinobacteria to degrade organic matter and thus participate in cell wall lysis, decomposition of lignin, cellulose and chitin. For example, the synthesis of cellulases, chitinases, and xylanases by *Micromonospora* allows the utilization of organic matter in bottom sediments. Peroxidase DypB from Actinobacteria strain *Rhodococcus jostii* was the first identified bacterial lignin-oxidizing enzyme. Importantly, the conversion of lignin from plant biomass to renewable chemicals is an emerging issue in biorefineries [2]. Therefore, exploring new microbial sources of biologically active substances is crucial.

Different strains of Actinobacteria produce oxidoreductase enzymes such as carbon monoxide dehydrogenase synthesized by *Streptomyces thermoautotrophicus*; laccase synthesized by *Thermobifida fusca* and alditol oxidase synthesized by *Acidothermus cellulolyticus*. The genus *Rhodococcus* produces a wide range of enzymes, such as oxygenases, peroxidases, phenolhydrolases, nitrilhydratases, dehydrogenases, alkylsulphatases [3]. *Frankia,* for instance, are nitrogenase producers and can form nodules in *Leguminosae*. Symbiosis enables assimilable nitrogen to be provided to plants and makes the bacteria a crucial factor in the circulation of this element in nature [1]. Moreover, some strains in symbiosis with plants can produce substances that support the resistance of these plants to bacteria and fungi.

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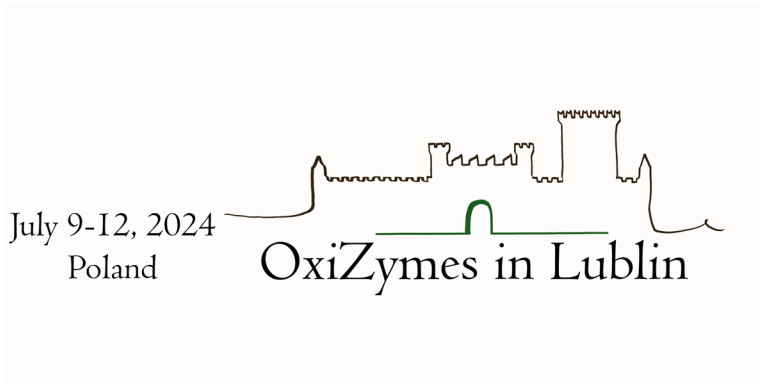
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NOTES

TUE

WED

THU

FRI

Tuesday, July 9th			Wednesday, July 10th			Thursday, July 11th			Friday, July 12th		
			8.30	REGISTRATION							
			9.00 OPENING						9.00	*Applications*	Doris Ribitsch
			9.00	Opening remarks		9.00	Frank Hollmann		9.20		Vânia Brissos
			9.20	Gustav Berggren		9.50	*Protein Engineering*	Omer Yehezkeli	9.40		Roland Ludwig
			10.10	*RedOx*	Gianantonio Battistuzzi	10.10		Dirk Tischler	10.00		Katja Koschorreck
			10.30		Ian Coupez	10.30		Ruben Walter	10.20		Mario De Simone
			10.50 COFFEE BREAK						10.40		Kota Takeda
			11.20	*RedOx*	Sebastian Gritsch	11.20	*Protein Engineering*	Clemens Peterbauer	11.00	CLOSING REMARKS & LUNCH	
			11.40		Mariusz Trytek	11.40		Feilong Li			
			12.00		Matteo Damian	12.00		Yuki Yashima			
			12.20		Ronald P. de Vries	12.20		Giuliano Sciara			
			12.40		Radka Koncitikova						
			13.00 LUNCH								
			14.30	*New Oxizymes*	Lígia O. Martins	14.30	POSTER SESSION *The best poster competition*				
			14.50		Caio de Oliveira Gorgulho Silva						
			15.10		Marcel Zámocký						
15.00-18.00	REGISTRATION		15.30		Hongbo Zhao						
16.00	Kiyohiko Igarashi		15.50	COFFEE BREAK		16.00-18.00	TOUR AROUND LUBLIN *City of Inspiration*				
16.45	*BioToP Session*	Anja Kostelac									
17.00		Lidiia Tsvik	16.15	Sophie Vanhulle Lecture							
17.15		COFFEE BREAK	17.00	Wiesław Gruszecki							
17.45		Wiktoria Piątek-Gołda									
18.00		Gaurav Patil	17.30	Welcome Cocktail and Meeting of The Scientific Committee							
18.15		Urban Leitgeb				20.00	GALA DINNER "Hotel Victoria" *The Best Poster Award*				