Ligno Biotech

TOULOUSE 2024

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On the conference

Replacing fossil carbon with renewable resources is essential to achieve climate neutrality goals. In this context, lignocellulose which is the second most abundant source of renewable carbon on Earth is the most promising feedstock.

Over the years, the LignoBiotech symposium has strengthened the scientific community involved in lignocellulosic biomass valorization. It has enabled a wide range of expertise to be shared at the various LignoBiotech sessions held in Reims, France (2010), Fukuoka, Japan (2012), Conception, Chile (2014), Madrid, Spain (2016), Helsinki, Finland (2018) and Vancouver, Canada (2022).

After 14 years of traveling the world, LignoBiotech 2024 brings our symposium back in France, in Toulouse. Located in the south of France, the Occitanie region is one of the most important agricultural regions in France, and Toulouse is the third largest French city dedicated to higher education and research with a strong focus on biotechnologies and the Bioeconomy.

LignoBiotech 2024 focusses on the latest biotechnological advances in biomass valorization, using all fractions of biomass -cellulose, hemicelluloses, extractives and lignin- to produce biomaterials, biofuels and fine chemicals. Particular emphasis will be placed on recent advances in biomass characterization in order to establish a link between biomass properties and promising bioproducts and biomaterials. LignoBiotech 2024 will highlight new opportunities for exploring and exploiting microbial and enzymatic biodiversity, using synthetic biology and artificial intelligence, and will address the sustainability of emerging and developed applications of lignocellulosic biobased products and services that meet the future challenges of developing a global Bioeconomy.

Topics

- Linking biomass molecular structure to bioproducts and biobased materials
- Advances and cutting-edge technologies for enzyme discovery and engineering
- Modeling and Artificial Intelligence contribution to lignocellulose valorization
- Bioconversion using single strains, co-cultures or synthetic microbial consortia
- Circular bioeconomy: valorizing all lignocellulose fractions
- Sustainable processes : LCA for lignocellulose bioconversion

Key figures

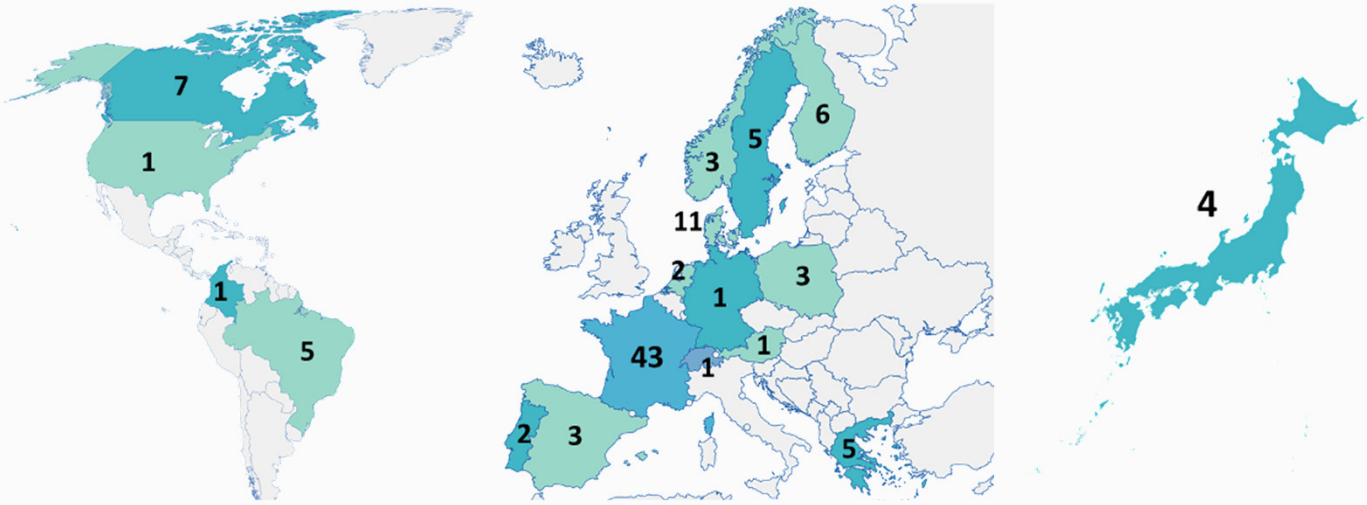
7th
Edition of a major international event dedicated to lignocellulose biotechnologies

6
Invited speakers

3
Conference days

103
International scientists

Participants



Conference chairs

Claire Dumon[1]

Claire Dumon is a senior scientist at INRAE. Within CIMES team in Biocatalysis department of TBI, she leads the SysCarb group dedicated to the characterization of microbial protein systems (carbohydrate active enzymes and transporters) that degrade polysaccharides. Her main topic focuses on understanding enzymes synergies which involves structure-function characterization, enzyme engineering and protein-carbohydrate interactions.

Guillermina Hernandez-Raquet[1]

Guillermina Hernandez-Raquet is senior scientist at INRAE, based at TBI. She holds a PhD in Biotechnology from INSA Toulouse. Her research focuses on the biodegradation and valorization of recalcitrant organic resources, in particular lignocellulose and lignin, through the production of valuable compounds. Her group is particularly interested in understanding microbial interactions in consortia involved in bioconversion processes, using multi meta-omics technologies.

Jean-Guy Berrin[2]

Jean-Guy Berrin is a senior scientist at INRAE, based at the BBF lab in Marseille. He leads the BOOST team, which focuses on investigating the biological roles of fungal carbohydrate active enzymes (CAZymes) in the degradation and modification of recalcitrant polysaccharides and synthetic polymers. The team brings together expertise in glycobiology, enzyme engineering, and fungal biotechnology.

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[2] BBF INRAE/ Aix-Marseille University, FR

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Local organizing committee

LignoBiotech 2024 is organized by Toulouse Biotechnology Institute – TBI

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Cédric Montanier – INRAE researcher, TBI, Toulouse

In collaboration with

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Sofiène Abdellaoui – Assistant professor, FARE – INRAE-, University of Reims

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US Department of Agriculture, USA

Regis Teixeira
Universidad de Concepción, Chile

Introductory talk

Pierre-Benoit Joly
Head of Occitanie –Toulouse
INRAE Center

Pierre-Benoit Joly, a Science and Technology Studies (STS) scholar, is Senio scientist at the National Institute of Research for Agriculture, Food and Environment (INRAE) in France. He has been the Director of the IFRIS and of Labex (Laboratory of Excellence) SITES from 2009 to 2014, and the founding director of LISIS, a research Unit based at Université Gustave Eiffel from 2015 to 2019. Since January 2020, he is the Chair of the INRAE Center Occitanie-Toulouse. His research is devoted to the study of co-production of knowledge and social order. Drawing on a number of empirical studies on the interactions between science, democracy and the market, the aim is to analyze the contemporary transformations of scientific public sphere and new modes of governance of innovation and risk. Most recently, he has focused on the analysis of research impact and on the transformations of innovation processes and innovation policies. Dr. Joly has published six books, coordinated four special issues of international journals and more than 120 articles or book chapters.

He has lectured in various higher education organization, including the Ecole des Hautes Etudes en Sciences Sociales (EHESS) and Sciences Po. He is a member of the French Academy of Agriculture, and of the French Academy of Technology.

From promises to impact:

The challenges of research on bioeconomy

Research and innovation are crucial for addressing current grand societal challenges. The shift from fossil-based economy to bio-based economy is among the major transformations to be achieved for addressing the challenges of both climate change and scarcity of natural resources. Such a shift is on the agenda of major public and private organisations since the early 70's. However, the actual transformations are not as important as expected. This leads to reflect on changes in research and innovation policy that are needed for better contributing to desired changes. In this talk, I will draw on current initiatives such as mission oriented innovation policies and impact oriented research management to explore the conditions and possibilities of such changes.

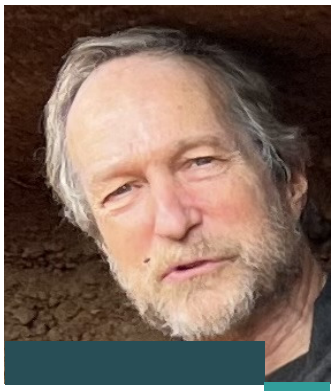
Invited speakers

Ingo Burgert

Ingo Burgert studied Wood Science and Technology at the University of Hamburg, Germany, followed by doctoral studies at the same university. Afterwards, he was a postdoc at the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria. From 2003 to 2011, he was a research group leader at the Max Planck Institute of Colloids and Interfaces, Potsdam, Germany. Since 2011 he has been a professor of Wood Materials Science at ETH Zurich, Switzerland, and a group leader at Empa, Dübendorf.

Jane W. Agger

Jane W. Agger is Associate Professor at the Technical University of Denmark (DTU), in the department of Biotechnology and Biomedicine. She obtained her PhD from the same institution in 2011. After a Post Doc at the Norwegian University of Life Sciences, she returned to DTU in 2015 to work on the discovery and novel characterization of glucuronoyl esterases and their activity towards ester-linked lignin-carbohydrate complexes. She is specialized in enzymes active towards lignocellulose and oxidative enzymes like laccases, polyphenol oxidases, unspecific peroxygenases and LPMOs. She entered tenure track in 2019 and has recently formed a new research group, Lignin Biotechnology where she continues to aim at understanding lignin active enzymes.



Michael Crowley

Michael Crowley is a principal scientist and Center Director of the Renewable Resources and Enabling Sciences Center, joining NREL in 2007 to develop a simulation and theory group. He has been Principal Investigator for multiple modeling projects in enzyme digestion, metabolic modeling, plant cell wall structure, reactor and process design, and multiple other collaborative projects in biofuels and computational chemistry research. He is a developer of both CHARMM and Amber molecular dynamics software for biological macromolecular simulation. Mike received his Ph.D. in Physical Chemistry from University of Montana, post-doctoral fellowship at Brandeis University.

Invited speakers

Lorie Hamelin

Lorie Hamelin is Chair Professor INRAE within Sustainable Bioeconomy Transitions in the Toulouse Biotechnology Institute (TBI) of INSA-Toulouse. Her research aims to design just and sustainable transition roadmaps towards low fossil Carbon economies, to quantify the associated environmental consequences of these roadmaps, and understand their performance dependencies. Counting with 15y of research experience in Canada, Denmark, Poland and France, Dr. Hamelin developed early methods for life cycle assessments of land use changes, biogas & overall manure management systems. Dr. Hamelin was awarded the prestigious "Make Our Planet Great Again" research grant from the French presidential climate call in 2017 to work in TBI and is involved in EU projects ALIGNED and NEGEM.

Michael O'Donohue

Michael O'Donohue works for INRAE, France's National Institute for Research on Agriculture, Food and the Environment. He holds a PhD in protein biochemistry from the University of Portsmouth (UK) and possesses extensive research experience in enzyme engineering and biotechnology. From 2016-2024 Michael was head of INRAE's TRANSFORM division. Since January 2024, Michael is Scientific Director of EXPLOR'AE, a national program that funds high-risk/high-gain research. In 2014, Michael launched a European initiative aimed at federating European capacity and developing a more systemic approach to research in biotechnology and biomanufacturing. Since then, this initiative has grown into IBISBA, a distributed European Research infrastructure that gained ESFRI recognition in 2018.

Sabina Leanti La Rosa

Sabina Leanti La Rosa received her PhD in Molecular Microbiology from the Norwegian University of Life Sciences (NMBU) in 2014. She then trained with Dr Barbara E. Murray at the University of Texas Health Science Center in Houston (USA), investigating clinically relevant pathogens. She moved back to Norway to begin work on gut microbiome, focusing on the enzymatic mechanisms through which gut commensals degrade food and feed components. Since 2022, she is an Associate Professor in Microbiology at the Norwegian University of Life Sciences. Sabina's current research applies multi-omics approaches and enzymology to investigate mechanisms by which gut commensals utilize complex glycans, including hemicellulosic substrates and food additives.

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Programme

MONDAY, 14th OCTOBER	
16:00 - 18:00	Arrival and Registration
18:00 - 18:30	Welcome by the chairs **Claire Dumon, Jean-Guy Berrin and Guillermina Hernandez-Raquet** Introductory talk by **Pierre Benoit Joly, Head of INRAE Occitanie-Toulouse Center**
18:30 - 20:00	Welcome cocktail

TUESDAY, 15th OCTOBER	
	Linking biomass molecular structure to bioproducts and biobased materials Maija Tenkanen (University of Helsinki, Finland) and Michael Crowley (NREL, USA)
9:00 - 9:40	**Keynote: Ingo Burgert ETH,** Zurich, Switzerland *Biobased Materials – Potentials and Challenges*
9:40 - 10:00	**Fabienne Guillon,** INRAE, BIA, Nantes, France *In situ imaging of LPMO action on plant tissues by mass spectrometry*
10:00 - 10:15	**Flash Presentations**
	Morten Rese, Norwegian University of Life Science: *Detailed characterization of hardwood and softwood lignin conversion by a brown-rot basidiomycete* **Koar Chorozian,** National Technical University of Athens: Enhancing Bio-based Material Production: *Characterization of a New LPMO from Fusarium oxysporum for Cellulose Extraction and Functionalization* **Christian Donohoe,** University of Copenhagen: *The oxidative effects of C1 or C4 - acting lytic polysaccharide monooxygenases on the production of nanofibrillated-cellulose from sugar-beet pulp*
10:15 - 10:50	Coffee
10:50 - 11:10	**Owen Mototsune,** University of Toronto, Canada *Applying Oxidative Enzymes to Make Fully Bio-based Chitosan Gels*
11:10 - 11:30	**Gabriel Paës,** INRAE-FARE, Reims, France *Dynamical assessment of fluorescent probes mobility in poplar cell walls reveals nanopores govern saccharification*
11:30 - 11:50	**Estelle Bonnin,** INRAE-BIA, Nantes, France *Tomato stem-reinforced composites: Biobased and biodegradable materials for a virtuous circular approach in the horticulture sector*
11:50 - 12:10	**Flash Presentations**
	Scott Mazurkewich, Chalmers University of Technology: *Understanding enzyme-substrate interactions and biological roles in Carbohydrate Esterase Family 15* **Edwige Audibert,** INRAE - Reims University: *Impact of structural and morphological features of industrially steam exploded woody biomass on bioethanol production* **Pedro A. Martins,** Technical University of Denmark: *The effects of glucuronoyl esterase activity on untreated lignocellulosic biomass* **Jonas Thomsen**, University of Copenhagen: *Applying biotech approach to unravel recalcitrance of peat*
12:10 - 13:20	**Lunch**
13:20 - 14:30	**Poster session**

Advances and cutting-edge technologies for enzyme discovery and engineering

Kiyohiko Igarashi (University of Tokyo, Japan) and Clemens Peterbauer (BOKU University, Austria)

14:30 - 15:10	**Keynote: Jane W. Agger DTU,** Denmark *Polyphenol oxidases as new biocatalysts for oxy-functionalization of lignin*
15:10 - 15:30	**Edita Jurak,** University of Groningen, The Netherlands *Assigning function of enzymes within genetic clusters*
15:30 - 15:50	**Meera Christopher,** Chalmers University, Gothenburg, Sweden *Bioprospecting filamentous fungi for robust cell wall degrading enzymes*
15:50 - 16:05	**Flash Presentations**
	Line Korte Martinsen, Technical University of Denmark: *Examining the interactions of plant biomass and microbial expansins -The hand grenade in lignocellulosic biorefinery?* **Anna JVC Brilhante** LNBR-University of Campinas: *Structural insights into a novel aldehyde-dehydrogenase active on cinnamaldehydes related to monolignols* **Simone Tarp Sunding,** Technical University of Denmark: *Unspecific peroxygenases and their oxidative activity towards lignin*
16:05 - 16:40	Coffee
16:40 - 17:00	**Ligia O. Martins,** Universidade Nova de Lisboa, Portugal *Enhancing the Value of Lignin-derived Chemicals through Tailored Bacterial Laccases*
17:00 - 17:20	**Iker Pardo Larrabeiti,** INSA, TBI, Toulouse, France *Investigating the spatial configuration of glycoside hydrolase multienzyme complexes on Plant Cell Wall breakdown*
17:20 - 17:40	**Maija Tenkanen,** University of Helsinki, Finland *Novel monolignol acting CAZy AA3_2 family oxidoreductases from plants*
17:40 - 18:00	**Wade Abbott,** Agriculture and Agri-Food Canada *Using combinatorial glycomic and molecular tools to inform microbial function in the ruminant gut microbiome*
18:00 - 19:30	**Poster session & beers**

WEDNESDAY, 16th OCTOBER

Modeling and Artificial Intelligence contribution to lignocellulose valorization

Ligia O. Martins (Universidade Nova de Lisboa, Portugal) and Evangelos Topakas (University of Athens, Greece)

9:00 - 9:40	**Keynote: Michael F. Crowley,** NREL, USA *Modeling the cell wall structure and machine learning for upgrading lignocellulosic intermediates to value-added polymers*
9:40 - 10:00	**Kiyohiko Igarashi,** University of Tokyo, Japan *Artificial Intelligence classifies white- and brown-rot fungi according to the number of the genes encoding Carbohydrate-Active enZyme families*
10:00 - 10:20	**Angel T. Martinez,** CSIC, Spain *Evolutionary evidence on oxidative attack of lignin at a solvent-exposed tryptophan in ligninolytic peroxidases*

10:20 – 10:50	**Coffee**
10:50 – 11:10	**Xuebin Feng,** University of Toronto, Canada *Engineering Amine Transaminase for Hemicellulose Amination*
11:10-11:30	**Susana Camarero,** CSIC, Spain *In vitro and in silico studies for laccase design*
11:30-11:40	**Flash Presentations**
	André Taborda, Universidade Nova de Lisboa: *Unveiling catalytic and structural properties of new bacterial carbohydrate oxidases* **Clemens Peterbauer,** BOKU University Vienna: *Characterization of the activity of dye-decolorizing peroxidases on O-glycosides and lignocellulose*

Bioconversion using single and co-cultures or natural and synthetic microbial consortia

Wade Abbott (Agriculture and Agri-Food, Canada) and Taina Lundell (University of Helsinki, Finland)

11:40 – 12:20	**Keynote: Sabina Leanti La Rosa,** Norwegian University of Life Sciences, Norway *Microbial processing of lignocellulosic polysaccharides in gut systems*
12:20 - 13:30	Lunch
13:30 - 15:00	**Poster session**
15:00 - 15:20	**Nicolas Vita,** CNRS – Aix Marseille University, Marseille, France *How to handle lignin in absence of oxygen: a bacterial lesson?*
15:20 - 15:40	**Tina Rise Tuveng,** Norwegian University of Life Science, Norway *Lignin utilization by bacteria in an anerobic environment?*
15:40 - 16:00	**Robert Röllig,** INRAE-Aix Marseille University, Marseille, France *Fungal wood decay under anoxia*
16:00 - 16:20	**Johan Larsbrink,** Chalmers University of Technology, Sweden *Microbial strategies for degradation of spruce bark*
16:20 - 16:40	**Flash presentations**
	Lison Degeilh, INRAE-BBF-Aix-Marseille University: *Characterization of a novel cellulosomal enzyme reveals an unusual fold among cinnamoyl esterases* **Etienne Pujos,** TBI-INRAE-INSA-Toulouse: *Characterization of lignin degradation by specialized bacterial consortia derived from termite gut* **Cristina Gonzalez Rivero,** Bavarian State Research Center for Agriculture: *Development of an agricultural-residue-based medium for cultivating anaerobic fungi*
16:40 - 18:30	**Poster session**
19:00 - 23:30	**Gala dinner Hôtel Dieu**

THURSDAY, 17th OCTOBER

Circular bioeconomy: valorizing lignocellulose residues and its lignin fraction

Adèle Lazuka (VERI, France) and Miia Mäkelä (Aalto University, Finland)

9:00 - 9:40	**Keynote : Michael O'Donohue,** INRAE - TBI, France *Trends in biotechnology & biomanufacturing: a European perspective.*
9:40 - 10:00	**Caroline Rémond,** INRAE- FARE Reims University, Reims, France *Multi-steps biocatalytic strategy to produce a portfolio of biomolecules of interest from lignocellulosic biomass*
10:00 - 10:20	**Gijs Van Erven,** Wageningen University, The Netherlands *Miscanthus sinensis conversion by the white-rot fungus Ceriporiopsis subvermispora: Plant genotype - fungal strain harmonization for total-use cascading into constituent biopolymers*
10:20 – 10:50	**Coffee**
10:50 - 11:10	**Katja Salomon Johansen,** Meliora Bio, Denmark *The Meliora Bio biorefinery: Production of dietary fiber and ethanol from wheat straw*

Sustainable processes: LCA for lignocellulose bioconversion

Ingo Burgert (ETH, Zurich, Switzerland)

11:10 - 11:50	**Keynote : Lorie Hamelin,** INRAE TBI, France *Environmental performance of lignocellulosic biomass valorization: What did we learn from LCAs and what are the research challenges ahead?*
11:50 - 12:20	Announcements and closing session
12:20 - 13:30	Lunch

Departure

Abstracts

Oral and Flash communications

Linking biomass molecular structure to bioproducts and biobased materials

Biobased materials – Potentials and Challenges

Ingo Burgert[1,2]

[1]Wood Materials Science, Institute for Building Materials, ETH Zürich, 8093, Zürich, Switzerland
[2]WoodTec Group, Cellulose & Wood Materials Laboratory, Empa, 8600 Dübendorf, Switzerland

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Keywords: Wood, Functional materials, Hybrid Materials, Cell walls, Modification

Session 1: Linking biomass molecular structure to bioproducts and biobased materials.

Biobased materials, particularly those from lignocellulosic resources, have sparked a growing scientific interest given their renewable nature and CO_2 storage capacity. Wood, the most abundant natural lignocellulosic biomass on earth, provides manifold opportunities to utilize its excellent properties in a future bioeconomy. Besides common uses in construction and pulp and paper processing, the development of functional wood and wood-derived materials has gained increasing attention in the last decades [1]. One approach is to disintegrate the cell walls and make use of the isolated cell wall polymers, mainly nanocellulose and increasingly lignin. These bottom-up approaches assemble the individual building units in advanced manufacturing processes resulting in biobased materials with tailored structures and properties. Alternatively, top-down approaches use and retain the hierarchical structure of wood with its beneficial fiber directionality [2]. Both approaches have advantages and limitations making them favorable for different kinds of applications [3].

This talk focuses on the exciting opportunities arising from the modification and functionalization of wood by taking advantage of its hierarchical structure. Wood provides cell walls with a rigid molecular structure, anisotropic properties, and a high porosity at higher length scales. Its excellent mechanical properties make wood a highly suitable raw material for developing high-performance composites and hybrid materials. Tailored wood treatments, which ideally should follow green chemistry principles, can equip wood materials with new functions and improved properties. Several concepts for obtaining functional wood materials will be presented, addressing the potentials and limitations of making wood a key resource of a net zero society.

[1] Chen, C. et al. (2020) Structure–property–function relationships of natural and engineered wood, Nature Reviews Materials, 5, 642–666

[2] Berglund, L. A., Burgert, I. (2018) Bioinspired Wood Nanotechnology for Functional Materials, Advanced Materials, 30,1704285

[3] Keplinger, T., Wang, X., Burgert, I. (2019) Nanofibrillated cellulose composites and wood derived scaffolds for functional materials, Journal of Materials Chemistry A, 7, 2981-2992

In situ imaging of LPMO action on plant tissues by mass spectrometry

Amandine Leroy[1,2], Mathieu Fanuel[2], Marie-Françoise Devaux[2], Camille Alvarado[2], Sylvie Durand[2], Anouck Habrant[1], Sacha Grisel[3], Mireille Haon[3], Jean-Guy Berrin[3], Gabriel Paës[1], Fabienne Guillon[2]

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[3] INRAE, Aix Marseille Université, UMR1163 BBF, 13009 Marseille, France

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Keywords: Oxidative enzyme, cellulose, MALDI-MS imaging

Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes that oxidise polysaccharides in the presence of an electron donor and O2 or H2O2. Numerous studies have reported the synergistic action of LPMOs with other fungal carbohydrate-active enzymes for the degradation of lignocellulosic biomass [1], but few studies have attempted to analyse their behaviour at the tissue level [2, 3]. Here, we investigated the enhancing effect of an LPMO active on AA9 family cellulose on the performance of the cellulosic Celluclast® cocktail on raw and pretreated maize stalks, ground material and internode sections. To achieve this goal, we developed an innovative MALDI-MS imaging workflow to detect oxidised oligosaccharides released by LPMO at the cellular level on maize tissues. The use of this workflow allows direct imaging of LPMO action and provides insight into the spatial variation and relative abundance of oxidised and non-oxidised oligosaccharides. In addition, FTIR microscopy and Simon staining were used to map cell wall composition and cellulose accessibility in plant tissues. Overall, we were able to show that the interaction between LPMO and cellulases is influenced by the structural properties of the cell wall, which vary from tissue to tissue. Indeed, the targeted action of LPMO is influenced by the polymer organisation of plant cell walls.

[1] T. Tandrup, K. E. H. Frandsen, K. S. Johansen, J.-G. Berrin, and L. Lo Leggio, "Recent insights into lytic polysaccharide monooxygenases (LPMOs), Biochem Soc Trans, vol 46, no 6, pp 1431-1447, 2018
[2] B. Chabbert,A. Habrant, M.Herbaut, L. Foulon, V. Aguié-Béghin, S. Garajova, G. Paës, "Action of lytic polysaccharide monooxygenase on plant tissue is governed by cellular type" Scientific Reports, vol 7, no 1, 17792, 2017.
[3] H. Chang, N. Gacias Amengual, A. Botz, L. Schwaiger, D. Kracher, S. Scheiblbrandner, R. Ludwig, "Investigating lytic polysaccharide monooxygenase-assisted wood cell wall degradation with microsensors", Nature Communications, vol 13, no 1, 6258, 2022..

Detailed characterization of hardwood and softwood lignin conversion by a brown-rot basidiomycete

Morten Rese[1], Gijs van Erven[2,3], Romy Veersma[3], Gry Alfredsen[4], Vincent G. H. Eijsink[1], Mirjam A. Kabel[3] and Tina R. Tuveng[1]

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Keywords: Bioconversion, lignin, brown-rot, py-GC-MS, NMR spectroscopy

Wood-degrading brown-rot fungi primarily target carbohydrates, while lignin becomes partially modified and of potential interest for targeted lignin valorization[1]. Here, we report a comprehensive comparison of lignin conversion by a brown-rot basidiomycete, *Gloeophyllum trabeum*, growing on a hardwood and a softwood substrate. By harnessing the latest advancements in analytical methodologies, we show that *G. trabeum* not only degrades polysaccharides efficiently and selectively but may also remove more lignin from wood than previously reported. Structure-wise, brown-rotted lignin appeared substantially C_α-oxidized, O-demethylated, depleted in interunit linkages, and enriched in diagnostic substructures indicative of C_α-C_β, β-O and O-4 bond cleavages in the β-O-4 aryl ether linkage. These findings enhance our understanding of lignin conversion by brown-rot fungi, revealing previously unknown aspects of this process. Specifically, despite the well-documented differences in lignin structure between hardwood and softwood[2], *G. trabeum* attacks the same bonds in the lignin structures, resulting in similar chemical modifications regardless of the wood substrate. Furthermore, we show that *G. trabeum* enhances the antioxidant capacity of the lignin, and that the residual lignin can be separated into low- and high-molecular weight fractions with distinct properties. This highlights the biotechnological potential of brown-rot fungi for developing lignin-based antioxidant or resin products.

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[1] D. J. Yelle, J. Ralph, F. Lu, and K. E. Hammel, "Evidence for cleavage of lignin by a brown rot basidiomycete," *Environmental Microbiology*, vol. 10, no. 7, pp. 1844-1849, Jul. 2008.

[2] W. Boerjan, J. Ralph, and M. Baucher, "Lignin biosynthesis," *Annual Review of Plant Biology*, vol. 54, pp. 519-546, Jun. 2003.

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Enhancing Bio-based Material Production: Characterization of a New LPMO from *Fusarium oxysporum* for Cellulose Extraction and Functionalization

Koar Chorozian[1], Anthi Karnaouri[2*], Maria Dimarogona[3], Marijana Ponjavic[4], Jasmina Nikodinovic-Runic[4], Evangelos Topakas[1*]

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Keywords: Lytic Polysaccharide Monooxygenases, *Fusarium oxysporum*, Cellulose Functionalization, Wheat Straw, Bacterial Nanocellulose

Lytic polysaccharide monooxygenases (LPMOs) are increasingly recognized for their pivotal role in the breakdown of plant biomass, offering promising applications in biotechnology. This study focuses on the characterization of the AA9 LPMO from *Fusarium oxysporum* (*Fo*LPMO9A), which displays versatile regioselectivity with C1, C4, and C1/C4 oxidative cleavage patterns on cellulose substrates [1]. The research aims to harness the capabilities of *Fo*LPMO9A for biochemical and functional characterization, as well as its application for isolating and functionalizing cellulose on both micro and nanoscale levels, with nanocellulose being a green biomaterial with potential to replace plastics [2]. An enzymatic process was developed to isolate microscaled cellulose with increased crystallinity from OxiOrganosolv pretreated wheat straw biomass. Additionally, *Fo*LPMO9A was applied to functionalize bacterial nanocellulose, introducing a novel approach since LPMOs have not been previously used for bacterial nanocellulose functionalization. The efficacy of these approaches was validated through advanced nanocellulose characterization techniques and fluorescence dyes. The findings underscore the potential of employing LPMOs for enzyme-mediated isolation and functionalization of cellulose, providing a sustainable and efficient approach for producing high-added-value bio-based products from agricultural waste or fermentation products.

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Flash presentation
P5

The oxidative effects of C_1 or C_4 – acting lytic polysaccharide monooxygenases on the production of nanofibrillated-cellulose from sugar-beet pulp

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Keywords: LPMO, Nanocellulose, Sugar beet pulp, Green chemistry, Lignin

Lytic polysaccharide monooxygenases (LPMO) are an exciting class of enzymes that allow for controlled free-radical oxidation onto the cellulose and lignin of the plant cell wall. This study investigated how different classes of LPMOs (C_1 & C_4) can be used for the production of nanofibrillated cellulose from sugar beet pulp, a low lignin (< 5%) biomass by-product from agriculture, for use in adhesives or plastic films.

Sugar beet pulp strips were homogenised and then a one-pot procedure of digestion by a mix of various hemicellulases and pectinases, followed by oxidation by LPMOs. The cellulosic product was filtered out, washed, and then microfluidised to fibrillate the cellulose microfibrils. The resulting suspensions were characterised in terms of rheology, morphology and composition, and the direct effects of each LPMO evaluated.

In the future this project aims to apply complementary lignolytic enzyme systems such as laccase-LPMO to create sugar beet pulp nanocellulose with tuneable properties.

Applying Oxidative Enzymes to Make Fully Bio-based Chitosan Gels

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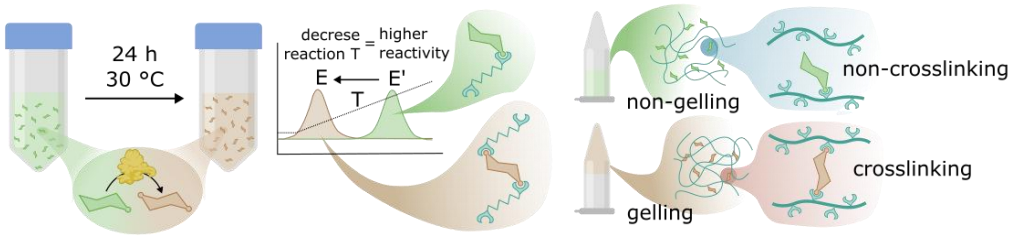
Keywords: oxidative enzymes; hemicellulose; lactose; chitosan; crosslinker



Carbonyl crosslinkers, such as formaldehyde, glyoxal, and glutaraldehyde, are used in textile, adhesive, and packaging applications, with megaton annual production volumes, but due to escalating concerns about their toxicity to human and environmental health and their non-renewable origins, less harmful bio-based alternatives are needed.

Six carbohydrate crosslinker candidates were produced by introducing carbonyl groups to lactose, galactose, xylose, and a mixture of xylooligosaccharides, using carbohydrate-active oxidoreductases: galactose oxidase from *Fusarium graminearum* (*Fgr*GalOx) and pyranose dehydrogenase from *Agaricus bisporus* (*Ab*PDH1). Their potential to react with hexamethylenediamine (HMDA) was then assessed using differential scanning calorimetry (DSC), revealing decreases in reaction temperature for four of the six carbohydrate crosslinkers—most notably a 34 °C decrease in reaction peak temperature for galactose oxidized by *Fgr*GalOx (72 °C) compared to unmodified galactose (106 °C). ATR-FTIR and XPS were used to identify imine crosslink formation and to investigate crosslinking reaction at temperatures relevant to crosslink formation.

Crosslinkers were then shown to form hydrogels when mixed with polyallylamine, with *Fgr*GalOx-oxidized lactose forming gels more effectively than all other crosslinkers, including glutaraldehyde, a conventional crosslinker. Imine bonds were verified in the gels produced using ATR-FTIR. Polyallylamine was then replaced with chitosan, an abundant polysaccharide found in crustaceans and fungi, to form fully bio-based imine gels. To our knowledge, this marks the first report of enzymatic methods being used to produce imine gels, demonstrating a proof-of-concept for enzyme biotechnology in the growing field of bio-based hydrogels. Further characterization of gels to investigate the rheological properties, self-healing ability, pH sensitivity, and biocompatibility is ongoing at the time of writing.

Dynamical assessment of fluorescent probes mobility in poplar cell walls reveals nanopores govern saccharification

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Keywords: Saccharification, Probes, FRAP, Modelling, Accessibility, Porosity

Improving lignocellulolytic enzymes' diffusion and accessibility to their substrate in the plant cell walls is recognised as a critical issue for optimising saccharification. Although many chemical features are considered as detrimental to saccharification, enzymes' dynam-ics within the cell walls remains poorly explored and understood. To address this issue, poplar fragments were submitted to hot water and ionic liquid pretreatments selected for their contrasted effects on both the structure and composition of lignocellulose. In addition to chemical composition and porosity analyses, the diffusion of polyethylene glycol probes of different sizes was measured at three different time points during the saccharification.

Probes' diffusion was mainly affected by probes size and pretreatments but only slightly by saccharification time. This means that, despite the removal of polysaccharides during saccharification, diffusion of probes was not improved since they became hindered by changes in lignin conformation, whose relative amount increased over time. Porosity measurements showed that probes' diffusion was highly correlated with the amount of pores having a diameter at least five times the size of the probes. Testing the relationship with saccharification demonstrated that accessibility of 1.3–1.7-nm radius probes measured by FRAP on non-hydrolysed samples was highly correlated with poplar digestibility together with the measurement of initial porosity on the range 5–20 nm.

Mobility measurements performed before hydrolysis can serve to explain and even predict saccharification with accuracy. The discrepancy observed between probes' size and pores' diameters to explain accessibility is likely due to biomass features such as lignin con-tent and composition that prevent probes' diffusion through nonspecific interactions probably leading to pores' entanglements.

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Tomato stem-reinforced composites: Biobased and biodegradable materials for a virtuous circular approach in the horticulture sector

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Tomato stems are the post-harvest biomass remaining from greenhouses after the period of tomato production. European production of tomato is rather stable (approximately 17 M tons in 2023) and stems and leaves represent approximately 1/5 of the usable production. The current method of growing tomatoes in greenhouses consumes many plastic accessories that are almost exclusively produced from petro-sourced and nondegradable polymers. The clips that fix the stem on the string and the bouquet holders that reinforce the peduncle represent about 500,000 pieces/ha and prevent the composting of plant by-product after greenhouses are emptied.

To develop a virtuous circular bioeconomy approach, tomato biomass was first evaluated as reinforcements for designing a range of degradable and biobased thermoplastic composite materials. The mechanical characterization shows that the tomato stem-reinforced materials can compete with existing formulations [1].

In a second step, the biodegradability of the tomato stem-reinforced materials was assessed using biochemical and imaging approaches along the enzymatic degradation by a mixture of cellulases and pectinases. Isolated or embedded in various matrix polymers, tomato stem particles remained sensitive to enzymatic degradation. Tomography analysis showed that the degraded samples exhibited a large increase in porosity, and that the biodegradation efficiency depends on the polymer tested [2].

This fully circular approach from waste to useful compounds for horticulture and market gardening is a promising way of upcycling tomato biomass, compatible with end-of-life composting.

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Understanding enzyme-substrate interactions and biological roles in Carbohydrate Esterase Family 15

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Keywords: CE15, glucuronoyl esterase, glucuronoxylan, lignin-carbohydrate complexes.

Glucuronoyl esterases (GEs) are α/β serine hydrolases from Carbohydrate Esterase family 15 (CE15) which cleave an ester linkage that connects lignin to glucuronyl xylan, an important linkage contributing to biomass recalcitrance. In recent years, we have been exploring the structure-function relationships of several CE15 members, particularly focused on bacterial members and those in species encoding multiple CE15 members [1]. Our research indicates that bacterial enzymes exhibit greater diversity compared to fungal members, often featuring large inserts near the active site, likely affecting substrate interactions and specificities. Crystal structures with ligands, as well as biochemical and computational studies, have further identified key residues and potential rate-limiting steps in the catalytic process [2]. While there is considerable evidence supporting the role of many CE15 enzymes as GEs in degrading lignocellulosic lignin-carbohydrate complexes (LCCs), our published work [3, 4] and further studies have revealed several unusual CE15 members in both fungi and bacteria. These CE15 proteins cannot be confidently identified as GEs due to their unique activity profiles, sequences, 3D structures, and genetic contexts. Presented will be our recent and ongoing work into gaining a better understanding of the family as a whole with particular attention given to possible new biological functions for these unusual CE15 members beyond decoupling lignin-glucuronoyl xylan linkages.

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Flash presentation
P9

Impact of structural and morphological features of industrially steam exploded woody biomass on bioethanol production

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Keywords: Lignocellulosic biomass; steam explosion; 2G bioethanol; multimodal characterization; correlations

Lignocellulosic biomass (LB) is a widely available renewable feedstock and can be used as an alternative to fossil resources to produce bioproducts such as biomaterials or biomolecules and especially second-generation (2G) bioethanol. Its complex structure leads however to biomass recalcitrance towards enzymatically catalysed biological conversion of its constituents: a pretreatment step is thus required to optimize production yields. Indeed, it induces beneficial physical (e.g. increased porosity) and chemical modifications (e.g. lignin removal) of LB favouring conversion of polysaccharides into 2G sugars.

To determine optimal steam explosion (SE) conditions, three wood species (oak, poplar and spruce) were pretreated with pilot-scale continuous SE at different severities by varying temperature and residence time. Fermentable sugars release and bioethanol production were monitored and data were modelled using an experimental design approach.

Multimodal characterization of pretreated wood was carried out to investigate the impact of structural and morphological modifications on the 2G sugars release. Physicochemical properties were interpreted using statistical analyses and correlations to establish the structure-properties relationships. Some features such as particle size, chemical composition, and lignin modifications were found to be related to bioethanol production increase, while others such as cellulose crystallinity and hydrophobicity had a negative impact during enzymatic saccharification. NMR approaches were also implemented to further study the impact of pretreatment at a molecular scale (cellulose and lignin ultrastructure) and at a morphological level (porosity) and relate it to the bioethanol production process efficiency.

Our study demonstrates the importance of the choice of starting LB materials as well as pretreatment conditions to maximize 2G bioethanol production from wood and the necessity to perform a multimodal approach to pinpoint the structural and morphological features having a critical impact on bioethanol production.

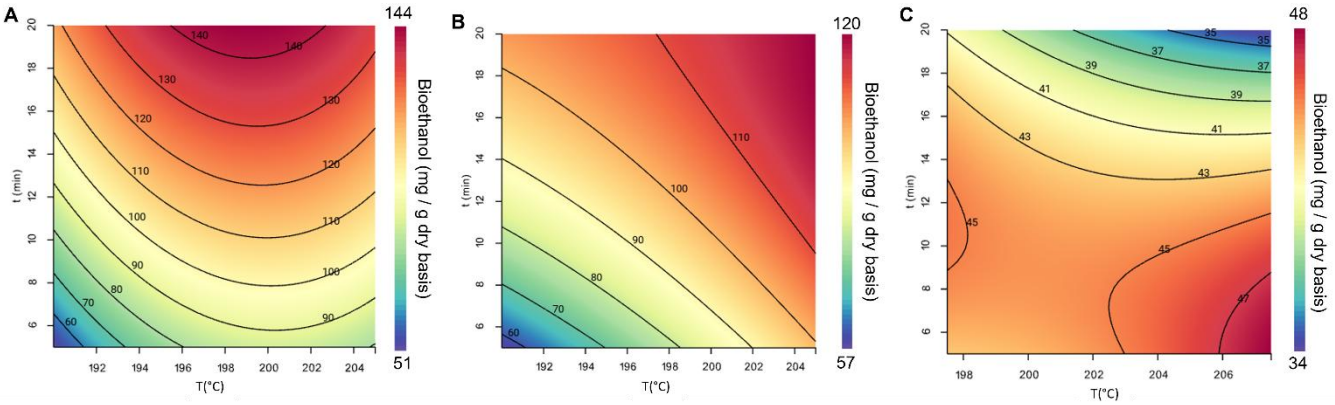

Figure: Bioethanol production response surfaces from oak (A), poplar (B) and spruce (C) pretreated with steam explosion at different severities.

Flash presentation
P11

The effects of glucuronoyl esterase activity on untreated lignocellulosic biomass

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Keywords: Glucuronoyl esterase; Lignin; Lignin-Carbohydrate Complex;

The biopolymers lignin, cellulose, and hemicellulose interconnect through a network of both covalent and non-covalent bonds to confer structural strength and resistance to microbial attacks. Lignin-Carbohydrate Complexes (LCCs) can exist as ester linkages between 4-O-Methyl-Glucuronoyl side chains on the glucuronoxylan backbone and gamma-positioned hydroxyl groups on the aliphatic portion of lignin, and they add to the recalcitrance of biomass. Glucuronoyl esterases (GEs) have demonstrated the ability to catalyze the hydrolysis of ester linkages between lignin and glucuronoxylan, and the removal of these esters by GEs is suggested to promote the disassembly of lignocellulose by loosening the interactions among the biopolymers in the plant cell walls. This study aimed at elucidating how GEs can enhance the accessibility of cellulases to cellulose fibers. Through a series of experiments, we investigated the synergistic potential of GEs and cellulases for increasing cellulose hydrolysis using untreated lignocellulosic biomass. To cover different types of biomass, the study encompassed hardwoods, softwoods, and cereals. Our results reveal a substantial increase in glucose yield upon the introduction of GEs alongside cellulases and a GH10 xylanase, underscoring the complementary roles of these enzymes in degrading the intricate lignocellulosic matrix. The cooperative action of GEs and cellulases leads to increased hydrolysis efficiency even in untreated lignocellulosic biomasses, attributed to the facilitated access of cellulases to cellulose microfibrils subsequent to ester bond cleavage. Ongoing investigations center on the extraction of lignin from biomass treated with GEs. We hypothesize that enzymatic treatment of biomass can not only enhance cellulose accessibility but also facilitate the extraction of lignin with properties closely resembling those found in its native state.

Applying biotech approach to unravel recalcitrance of peat

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Keywords: Sphagnum peat, recalcitrance, pretreatment, enzymatic saccharification, biomass characterization.

Sphagnum peat bogs make up a large pool of biologically bound carbon that has accumulated for thousands of years. Peat is defined as partially degraded organic matter and is believed to accumulate because of unfavorable conditions for microbial degradation, such as anoxic conditions, low pH, high concentration of phenols and polysaccharide composition. The cause of *Sphagnum* peat recalcitrance to degradation is unresolved, despite being of great importance for preservation efforts and climate models. Methods to study biomass recalcitrance have especially been developed in the biotech industry utilizing lignocellulosic biomass, such as sugar cane bagasse, corn stover and wheat straw for bio-based products, like ethanol. To investigate the resistance of *Sphagnum* peat, we applied methods developed for industrial processing of lignocellulose from higher plants, on gar-den peat (GP), which was used as a model substrate for peat. Hydrothermal pretreatment at temperatures ranging from 120 °C and 180 °C was applied and the pretreated material was analysed for mono- and oligosaccharide content. A commercial enzyme cocktail was used to evaluate the recalcitrance towards enzymatic saccharification in a laboratory setting. GP pretreated at 120 °C is completely inaccessible (fig. 1A), while 180 °C renders GP very accessible for the enzyme cocktail (fig. 1B**)**. However, a rapid decline in apparent enzyme efficiency was seen. This is in clear contrast to the relatively slow but steady conversion seen for pretreated wheat straw (fig. 1C),

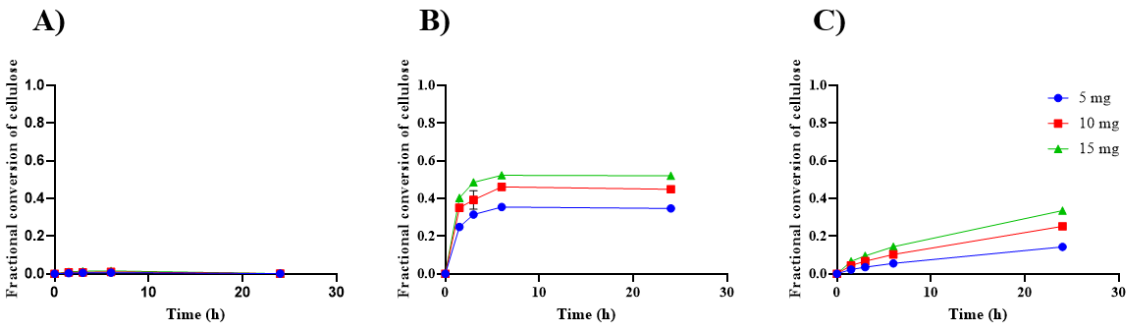

Figure 1. Effect of pretreatment temperature on GP saccharification progress curves. Duplicate samples of A) GP pretreated at 120 °C, B) GP pretreated at 180 °C and C) pretreated wheat straw was incubated for 24h with a commercial enzyme cocktail added in dosages of 5 mg / g DM (blue), 10 mg / g DM (red) and 15 mg / g DM (green) at 50°C, pH 5.2. The data are presented as averages ± standard deviation of duplicate sample.

Advances and cutting edge technologies for enzyme discovery and engineering

Polyphenol oxidases and their activity on lignin units

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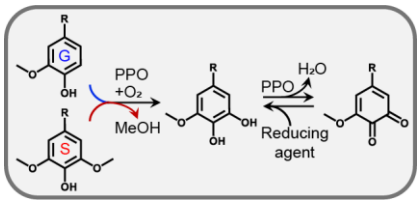
Keywords: polyphenol oxidase, lignin units, demethoxylation, diphenols,

Selective and precise oxidation of lignin and lignin-derived compounds is tremendously attractive to achieve in lignin processing. We present our latest results on how polyphenol oxidases (PPOs) may be the enzymes that can perform such unique modifications[1]. PPOs are coupled binuclear copper enzymes they are widespread in nature, and famous for their canonical tyrosinase activity; *ortho*-hydroxylation of tyrosine into the corresponding diphenol (monophenolase) followed by two-electron abstraction into the quinone (diphenolase). We describe a number of enzymes from a particular sub-group of PPOs known as short fungal PPOs, which do not have the canonical tyrosinase activity but rather selectively perform hydroxylation of guaiacyl-derived compounds (G-units) as those found in lignin. Additionally, we present the completely novel and rare ability of some of these short fungal PPOs to oxygenate syringyl (S)-type compounds. Assays with $^{18}O_2$ confirm that the conversion of S-units occurs via a combination of *ortho*-hydroxylation and *ortho*-demethoxylation with concomitant release of methanol. These special PPOs therefore have the ability to unify lignin-derived products from a mixture of G- and S-units into one type of methoxy-*ortho*-diphenols.

The PPOs further catalyse the two-electron abstractions from the diphenolic products (diphenolase activity) and form the corresponding methoxy-*ortho*-quinones. The reactive *ortho*-quinones tend to polymerize and LC-MS analysis reveals that polymerization happens with all the tested enzymes, yet we demonstrate that the presence of a reductant such as ascorbic acid can suppress the formation of quinones. The yield of diphenols vary both according to the substrates' *ortho* and *para*-substituents and with the exact PPO-variant. This allows the two activities (mono- and diphenolase) to occur either via a one-step mechanism or via two discrete reaction steps. Based on our findings we propose a new activity for short fungal PPOs, namely *O*-methoxy-phenolase activity.



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Assigning function of enzymes within genetic clusters

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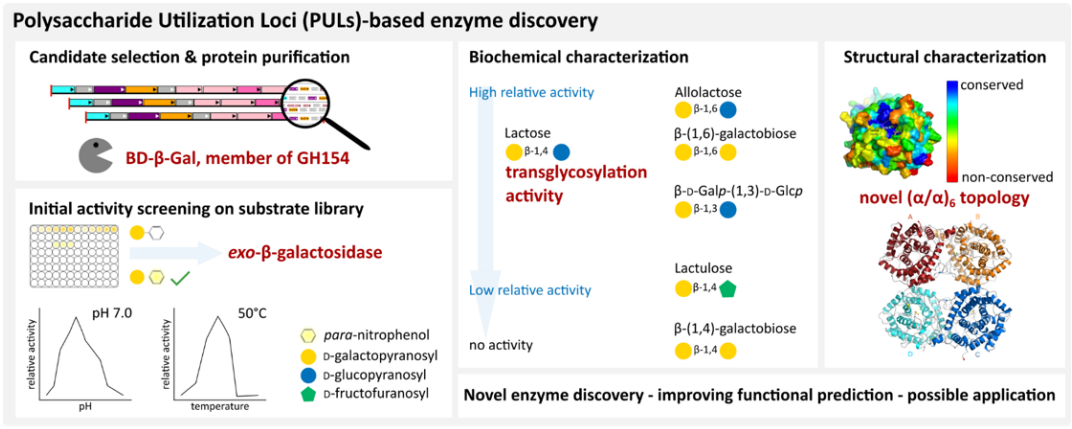
Keywords: polysaccharide utilization loci, carbohydrates, enzymes, unknown

In public genome databases, a substantial portion of genomic content, reaching up to 86%, lacks functional annotation [1]. Addressing this knowledge gap became important in order to enhance and sustain the predictive capabilities of these databases. Therefore, especially the identification and characterization of Proteins of Unknown Function (PUF) and DUFs become a critical endeavor [2]. Within this context, PULs proved to be an invaluable resources for the selection of targets [3]–[5]. These loci encode a rich diversity of proteins, including both PUFs and DUFs. Next to this, biochemical characterization of poorly characterized glycoside hydrolase families has potential for novel applications. While exploring PUFs, genetic makeup and DUFs may pose a higher level of risk due to their uncharted territory, the potential rewards in terms of advancing the understanding of their functions are substantial, making them promising candidates for in-depth functional characterization. Interestingly, for newly assigned glycoside hydrolase families, the functions reported, often, do not tell the whole story.

Figure 1. Scheme of novel enzyme characterization [5]



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BIOPROSPECTING FILAMENTOUS FUNGI FOR ROBUST CELL WALL DEGRADING ENZYMES

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Keywords: CAZymes; acidophilic; biorefinery; bark; genome

The natural biodiversity of microorganisms offers a vast reservoir of genetic diversity that can be exploited to discover stable, efficient or novel enzymes for biomass valorization. Extremophilic microbes, in particular, are known to harbor enzymes with unique biochemical properties and catalytic actions. To meet the demand for diverse biomass-derived products and enhance efficiency of biomass deconstruction, targeted bioprospecting is crucial. This involves identifying enzymes for various purposes, such as for extracting novel compounds from biomass (e.g., suberinases for fatty acid extraction from bark) or for improving existing enzyme cocktails (e.g., supplementation of LPMOs to cellulases).

Since filamentous fungi are known to encode extensive and diverse portfolios of CAZymes, we have explored extremophilic fungi from niche ecosystems for enzymes resilient to the inhibitory environments created during biomass pretreatments. We identified species of *Talaromyces* and *Penicillium* with esterases capable of releasing long-chain fatty acids (LCFAs), as demonstrated by their activity on model substrates. LCFAs are abundant in suberin- a major biopolymer in bark, and therefore, these *suberinases* hold promise for bark valorization. Moreover, the thermotolerant and acidophilic nature of these enzymes are favorable for applications on acid-pretreated biomass.

In case of biomass saccharification, studies with the thermophilic fungus *Thermothielavioides terrestris* LPH172 revealed the production of a consortium of lytic polysaccharide monooxygenases (LPMOs) with varying substrate specificities, that worked in tandem with hydrolases to effect lignocellulose degradation. Forestry-derived biomass like spruce wood are quite recalcitrant to enzymatic hydrolysis; as such, the identification of LPMOs that can create access points for hydrolases on both cellulose and hemicellulose is an important factor that can improve the saccharification efficiencies of such substrates.

Therefore, our bioprospecting endeavors aim not only to discover novel biorefinery enzymes, but also to understand the substrate-enzyme interactions that affect the rate and efficiency of lignocellulose depolymerization.

Examining the interactions of plant biomass and microbial expansins -The hand grenade in lignocellulosic biorefinery?

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Keywords: Expansin, Lignocellulose, Biomass, Synergy, Binding, Cellulase

Every year, gigatons of plant biomass is generated on earth, a sustainable process that captures and incorporates CO_2 into the fundamental components of the plant cell wall: cellulose and hemicellulose, and the aromatic polymer lignin. The properties of lignin are remarkable for future applications, but exploitation relies on more gentle and protective extraction from plant biomass. Lignin can substitute crude oil in different applications e.g. plastic, carbon fibre and battery production, and it has chemical properties applicable in pharmaceuticals, food and paint industries [1]. Inspired by nature, we study how enzymes and other proteins can facilitate gentle extraction of lignin.

Expansins are proteins without catalytic activity, and they are hypothesised to disrupt non-covalent bonds in the cell wall matrix thereby creating more physical space [2]. This allows water and enzymes to enter the matrix and increases substrate accessibility which enhances biomass conversion and possibly improve lignin fractionation. The expansin protein structure consists of two domains: a double-psi β-barrel (DPBB) related to glycoside hydrolase family 45 (GH45) but without catalytic activity; and the expansin specific carbohydrate binding module family 63 (CBM63) which binds cellulose. Loosenins only contain the DPBB.

We have recombinantly expressed a subset of expansins and loosenins from Neocallimastigomycetes and we investigate binding and mode of action of these proteins to cell wall constituents. Neocallimastigomycetes are rumen inhabitants of herbivores, which possess a significantly higher abundance of carbohydrate active enzymes (CAZymes) than other fungal species [3]. We focus on studying substrate binding, capability of reducing substrate crystallinity and synergism with other lignocellulosic active enzymes on model and complex plant substrates. Synergy between expansins and a minimal cellulase cocktail is currently the focus area of the study. Additionally, we are studying protein NMR for structure elucidation and mapping of residues relevant in binding.

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Structural insights into a novel aldehyde-dehydrogenase active on cinnamaldehydes related to monolignols

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Keywords: structural enzymology, enzyme rational design, substrate specificity

Lignin-related aromatics are potential renewable carbon sources for the biotechnological production of chemicals of industrial interest. Several metabolic pathways for their conversion into bioproducts have been described so far. However, the knowledge on enzymatic strategies for converting coniferaldehyde, *p*-coumaraldehyde, and sinapaldehyde are very limited. Recently, we identified an aldehyde-dehydrogenase (MolB) in *Xanthomonas citri*, sharing 30% sequence identity with $CalB_{HR199}$ from *Pseudomonas* sp. HR199, the first reported coniferaldehyde dehydrogenase. Despite their importance for lignin valorization, the structural basis for substrate specificity in these enzymes remains unclear. Here, we investigated the substrate profile and structure-function relationships of MolB using biochemical assays, structure prediction, molecular dynamics (MD), and rational design. Both MolB and $CalB_{HR199}$ are NAD^+-dependent dehydrogenases with higher activity towards coniferaldehyde. MD analysis and structural comparisons suggest that MolB residues R113 and E117 regulate substrate access and positioning in the active site. $CalB_{HR199}$, which has 20 times higher activity towards coniferaldehyde than MolB, served as a model for site-directed mutagenesis, leading to the replacement of MolB residues with their correspondent in $CalB_{HR199}$ (R113L and E117G), mimicking its more accessible active site. R113L mutation decreased enzyme's activity towards coniferaldehyde. Structural analyses indicated that R113 forms a hydrogen bond with the oxygen of the methoxy group of coniferaldehyde, suggesting that this residue plays an important role in coniferaldehyde recognition. Interestingly, the R113L mutation increased the enzyme's activity towards *p*-coumaraldehyde, which likely relates to the hydrophobic nature of leucine compared to arginine. R113L mutation did not impact enzyme's activity towards sinapaldehyde. E117G and R113L/E117G mutations significantly reduced activity towards all tested substrates when compared to WT. Thermal denaturation assays revealed lower melting temperatures for these variants (about 10 ºC decrease), and structural analysis indicated that the E117 residue might interact with R76 from an adjacent chain, likely contributing to tetramer stabilization. These evidences suggests that the E117G mutation disrupts MolB tetramer formation and negatively impacts enzyme activity. In summary, these findings enhance our understanding of the structure-function relationships of aldehyde-dehydrogenases active on cinnamaldehyde derivatives related to lignin. Further analyses are underway to better understand the factors underlying the observed results.

Unspecific peroxygenases and their oxidative activity towards lignin

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Keywords: Unspecific Peroxygenases, oxidative enzymes, lignin, biocatalysis, biomass conversion.

Unspecific peroxygenases (UPOs, EC 1.11.2.1) are heme-thiolate proteins exclusively found in the fungal kingdom. They belong to the family of oxidoreductases and efficiently catalyse the insertion of an oxygen atom from hydrogen peroxide into a diverse variety of substrates. These enzymes are especially abundant in lignocellulose-degrading fungi and are secreted during fungal delignification together with well-known lignolytic enzymes such as laccases and peroxidases.

UPOs are especially interesting for the enzymatic conversion of lignocellulose because they exhibit oxidative activity towards a wide variety of functional groups abundant in the lignin polymer, including aromatics, phenols, ethers, and methoxy groups. The aim of the project is to investigate and understand UPOs' ability to catalyse specific oxidative modifications on lignin or lignin derivatives, which could potentially increase the reactivity and applicability of lignin from plant biomass.

To achieve this, we heterologously expressed selected UPOs from the phyla Ascomycetes and Basidiomycetes in *Pichia pastoris*. These UPOs catalyse hydroxylation, demethoxylation, and one-electron oxidation on lignin substructures. In an online NMR study, we see indications that the UPOs form geminal diols and hemi-acetal intermediates. Our results reveal that UPOs originating from the same organisms display distinct ratios of peroxidase to peroxygenase activity, along with different pH dependencies. We aim to investigate how addition of hemin in the expression process affects the metal stoichiometry of the UPOs' active site and thereby the observed enzymatic activity and we will investigate the redox potentials of the individual UPOs by cyclic voltammetry to understand reaction preferences and substrate specificities. The overall goal is to understand the UPOs' ability to catalyse oxidative modifications on polymeric lignin and their potential synergistic effects with known lignocellulolytic enzymes.

Enhancing the Value of Lignin-derived Chemicals through Tailored Bacterial Laccases

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Keywords: Biocatalysis; Enzyme engineering; Biorefinery; Lignans; Natural Products

Enzymatic conversion of lignin phenolic platform chemicals offers a promising, eco-friendly approach to valorize lignin bio-wastes: the pulp and paper industry boasts an annual lignin production of approximately 50 million tons, but only around 1 million enter the chemical market. The challenge is to create cost-effective processes that minimize waste by fully utilizing lignin, converting it into a sustainable source for drop-in chemicals, polymers, and new functional materials.

Laccases, potent biocatalysts in lignocellulose biorefineries, hold great promise for converting lignin monomers into eco-friendly chemicals and materials. Despite a notable decrease in enzyme costs over the last twenty years, they remain a substantial expense in biorefining. Protein engineering helps to tailor enzymes to increase their catalytic efficiency. [1,2] In this study, we utilized directed evolution tools to improve > 400-fold the catalytic efficiency (k_{cat}/K_m) of a hyperthermostable bacterial laccase for lignin-related phenolics, including hydroxycinnamyl alcohols, cinnamic acid and vanillyl derivatives. We optimized biocatalytic oxidative processes at alkaline pH[3] to synthesize several lignans, such as syringaresinol, pinoresinol and diapocynin, among others, at a multigram scale, with very good to excellent yields (60%-100%), in most cases as single products, showing clear advantages over previously reported methods. Lignans are biologically active compounds with antioxidant, anti-inflammatory and antimicrobial properties, with applications in food, cosmetic and pharmaceutical industries.[4] Some are also suitable precursors of polymers.[5] They are natural products typically extracted from plants in time-consuming processes, underscoring the efficiency of enzymatic approaches. Our research indicates that the new enzyme is a promising option facilitating high yields of biologically active compounds through efficient, affordable, and environmentally friendly bioconversions.

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Investigating the spatial configuration of glycoside hydrolase multienzyme complexes on Plant Cell Wall breakdown

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Keywords: cellulase; xylanase; spatial organization; biomolecular welding tool; SAXS

To cope with the inherent complexity of plant cell walls and to extract oligosaccharides as carbon source, certain anaerobic microorganisms produce a wide range of glycoside hydrolases (GH) organized in a multienzyme complex: the cellulosome. Due to its intrinsic flexibility, studying the effects of the spatial orientations of the different enzymes within the cellulosome and the impact of these orientations on catalysis is very difficult. One approach to address this question is to lock the spatial proximity between GHs in different orientations and measure how each orientation modifies the activity. To do this, we use the biomolecular welding tool, composed of two small proteins, Jo and In [1]. These proteins spontaneously form an intramolecular isopeptide bond, offering a unique approach for geometrically freezing the spatial conformation between several GHs.

In this study, we propose to focus on three cellulosomal GHs from *C. thermocellum* (*Ct*Cel8A, *Ct*Cel9R, and *Ct*Xyn11A) to produce various combinations of multienzymatic complexes. Out of the 24 possible orientations for arranging the three enzymes via Jo/In, already 12 were successfully produced and purified. The enzymatic activities of each enzyme within the complexes were determined using small soluble substrates, while the overall activities of the complexes were determined using cellulose, arabinoxylan, wheat straw and wheat bran. Several analytical approaches (HPLC and mass spectrometry) were used to study the profile of products released. To discuss the close relationship between the topological differences created and the released product profiles, solution models of these complexes were generated using small-angle X-ray scattering (SAXS). We thus propose a global and systematic approach to address more generally the importance of spatial topology in multienzyme complexes for biocatalysis.

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Novel monolignol acting CAZy AA3_2 family oxidoreductases from plants

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Keywords: AA3, Arabidopsis, birch, monolignols, oxidoreductases

Oxidative enzymes targeting lignocellulosic substrates are presently classified into various auxiliary activity (AA) families within the Carbohydrate Active enZyme (CAZy) database. Key enzymes involved in the modification of lignin and other aromatic compounds are presently found in the AA1 family (laccases and multicopper oxidases), the AA2 family (lignin peroxidases, manganese peroxidases and versatile peroxidases), the AA3 subfamily 2 (aryl alcohol oxidases), and the AA4 family (vanillyl alcohol oxidases). Until now, research on FAD-containing oxidoreductases of the AA3 family has primarily focused on microbial sources and plant-derived AA3 family members are practically uncharacterized, although multiple AA3-encoding genes exist also in plant genomes. Plant AA3 sequences form a phylogenetically distinct group from microbial AA3 sequences and thus may encode enzymes with novel specificities.

In our recent screening, guided by sequence analysis and expression profiles, we identified a new AA3 family aryl alcohol dehydrogenase (*Bp*ADH1) in silver birch. The enzyme was produced in *Pichia pastoris* for biochemical characterization. *Bp*ADH1 showed a broad substrate specificity being able to oxidize various aryl alcohols, preferably cinnamyl alcohol and monolignols coniferyl alcohol and *p*-coumaryl alcohol converting them to corresponding aldehydes. However, sinapyl alcohol was a poor substrate. Benzoquinone was an effective electron acceptor, whereas oxygen was accepted poorly. Notably, *Bp*ADH1 also efficiently used laccase-generated phenoxy radicals from ferulic acid and *p*-coumaric acid as electron acceptors, whereas sinapic acid radicals were not preferred. Interestingly, Arabidopsis genome contains two *BpAdh1* orthologs and the corresponding proteins were produced heterologously and biochemically characterized. Both Arabidopsis enzymes also preferred cinnamyl alcohol, coniferyl alcohol and *p*-coumaryl alcohol as electron donors but one was an oxidase (*At*AAO1) using oxygen as an electron acceptor whereas other was a dehydrogenase (*At*AADH1) reducing hydroquinone. They also showed different localization.

Discovery and properties of novel AA3 family aryl alcohol oxidases/dehydrogenases from plants and potential pathways by which they may contribute in lignin modification and biosynthesis will be discussed in more detail on the present paper.

Using combinatorial glycomic and molecular tools to inform microbial function in the ruminant gut microbiome

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Keywords: Glycomics, Microbial ecology, Bioinformatics, Enzyme discovery, Structural polysaccharides

There is a knowledge gap regarding the factors that impede the ruminal digestion of structural polysaccharides within cell walls and if the rumen microbiota possess capacity activities to overcome these constraints. Innovative experimental methods have been developed to provide a high-resolution understanding of plant cell wall chemistries, identify higher-order structures that resist microbial digestion, and determine how they interact with the functional activities of the rumen microbiota. As a proof-of-concept, we have characterized the total tract indigestible residue (TTIR) from cattle fed a low-quality straw diet using two comparative glycomic approaches: ELISA-based glycome profiling and total cell wall glycosidic linkage analysis. We successfully detected numerous and diverse cell wall glycan epitopes in barley straw (BS) and TTIR and determined their relative abundance pre- and post-total tract digestion. Of these, xyloglucans and heteroxylans were of higher abundance in TTIR. To determine if the rumen microbiota can further saccharify the residual plant polysaccharides within TTIR, rumen microbiota from cattle fed a diet containing BS were incubated with BS and TTIR *ex vivo* in batch cultures. Transcripts coding for carbohydrate-active enzymes (CAZymes) were identified and characterized for their contribution to cell wall digestion based on glycomic analyses, comparative gene expression profiles, and associated CAZyme families. High-resolution phylogenetic fingerprinting of these sequences encoded CAZymes with activities predicted to cleave the primary linkages within heteroxylan and arabinan. This experimental platform provides unprecedented precision in the understanding of forage structure and digestibility, which can be extended to other feed-host systems and inform next-generation solutions to improve the performance of ruminants fed low-quality forages [1]. Our team has recently expanded beyond conventional feeds, studying the digestibility of seaweeds by rumen microbiota and exploring the potential for atypical feeds in the mitigation of climate challenges for livestock using similar research platforms. This approach has lead to discovery of enzymes

and pathways from the gastrointestinal microbiota of cattle and other ruminants with the capacity to metabolise polysaccharides from diverse seaweeds.

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Modeling and Artificial Intelligence contribution to lignocellulose valorization

Modeling and Machine Learning for Lignocellulose conversion to Value-Added Products

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Keywords: Lignocellulose, Conversion, Machine Learning, Molecular Modeling, Enzyme

The growth of a robust circular bioeconomy in which renewable and waste feedstocks are con-verted into recyclable value-added products is the aim of a host of collection of dedicated and talented scientists across the world. This presentation gives insight into the contributions of vari-ous computational, theoretical, and machine-learning approaches to enhance the experimental and manufacturing efforts to discover and economically produce new products that are at least as good as existing non-recyclable products made from non-renewable feedstocks. PolyID is a machine-learning approach to predicting properties of polymers from any monomer, such as ones we might derive or produce from waste and renewable sources. For example, we can apply its predictive power to all metabolic intermediates or pyrolysis oil components as a first step in developing a manufacturing process that will enhance the production of monomers for value-added recyclable products in food packaging, textiles, membranes, and structural materials. Machine learning also has allowed the discovery of multiple enzymes for conversion of lignocellulosic matter and recy-cling of plastics, mainly through innovative training and searching the protein database. We have several examples of success in enhancing conversion enzymatic processes through machine-learning-aided research. Molecular modeling approaches have been instrumental in guiding en-zyme design by advanced simulation of active sites in multiple biomass and plastics. So much of renewable resource is based on the plant cell wall, we have put considerable effort into under-standing the structure of the cell wall. Our aim is to understand how the polymers in a cell wall are arranged, what the forces holding them together is, what makes them so strong, and how we can modify them to be more economical as a source of molecules for our circular bioeconomy.

Artificial Intelligence classifies white- and brown-rot fungi according to the number of the genes encoding Carbohydrate-Active enZyme families

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Keywords: Lytic polysaccharide monooxygenase; wood-rotting fungi; Carbohydrate-Active enZymes; machine learning; random forest algorithm

Wood-rotting fungi play an important role in the global carbon cycle because they are only known organisms that digest wood, the largest carbon stock in nature. Wood-rotting fungi are categorized as either white- or brown-rot modes based on the coloration of decomposed wood. The process of classification can be influenced by human biases. In the present study, we used linear discriminant analysis (LDA) and random forest (RF) machine learning algorithms to predict white- or brown-rot decay modes from the numbers of genes encoding Carbohydrate-Active enZymes (CAZymes) with over 98% accuracy [1]. Unlike other algorithms, RF identified specific genes involved in cellulose and lignin degradation, including auxiliary activities (AA) family 9 lytic polysaccharide monooxygenases, glycoside hydrolase family 7 cellobiohydrolases, and AA family 2 peroxidases, as critical factors. These findings not only aid in the classification of wood-rotting fungi but also facilitate the identification of the enzymes responsible for degrading woody biomass. This study sheds light on the complex interplay between genetic information and decay modes and underscores the potential of RF for comparative genomics studies of wood-rotting fungi.

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Evolutionary evidence on oxidative attack of lignin at a solvent-exposed tryptophan in ligninolytic peroxidases

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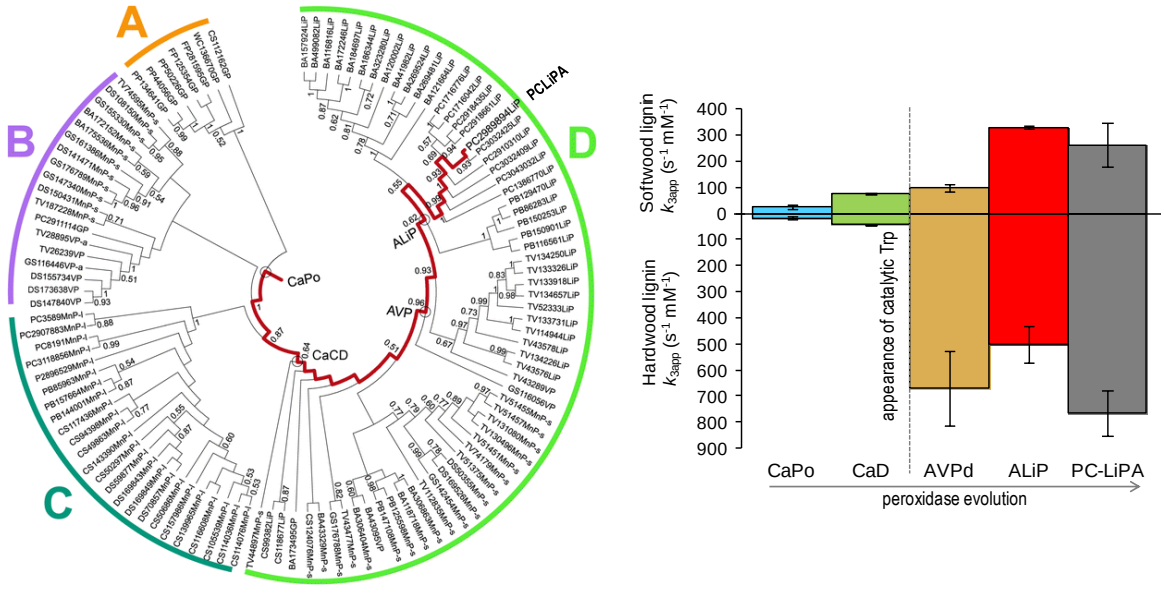
Keywords: fungal ligninolytic peroxidases; long-range electron transfer; enzyme mechanisms

A key finding to understand lignin biodegradation was the ability of lignin peroxidase (LiP) and versatile peroxidase (VP) to delocalize one oxidation equivalent from the buried heme to a surface tryptophan residue. In this way, the resulting radical is able to interact with the bulky lignin polymer, and transfer electrons to heme using a long-range pathway. The catalytic nature of the surface tryptophan had been demonstrated by directed mutagenesis using simple lignin models, but similar steady-state studies cannot be performed with lignin due to difficulties to estimate initial oxidation rates. However, it is possible to follow electron transfer from soluble (sulfonated) lignins from the "peroxidase side" (i.e. by estimating the constants for enzyme reduction under stopped-flow conditions) as shown in this study.

Moreover, the possibility to reconstruct ancestral proteins permits nowadays to directly identify the evolutionary change responsible for every new enzyme property. Here, four LiP ancestors (red path in peroxidase main clade D, Figure *left*) were resurrected (by sequence reconstruction, heterologous expression in *Escherichia coli*, and *in vitro* activation) and their ability to transfer electrons to lignin were evaluated together with extant *Phanerochaete chrysosporium* LiP (PC-LiPA). As shown in the Figure (*right*) a relationship exists between the rate-limiting constant in lignin reactions (k_{3app} from above stopped-flow analyses) and the presence of the catalytic tryptophan in AVPd, ALiP and PC-LiPA (absent from the previous CaPo and CaD ancestors).[1] This correlation was stronger for hardwood lignosulfonate, suggesting that the surface tryptophan was introduced in fungal peroxidases to better act on the more complex lignin developed by angiosperms. The appearance of a surface tryptophan paralleling the increase of peroxidase activity on lignin provides evolutionary evidence on the role of this residue in ligninolysis.



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Engineering Amine Transaminase for Hemicellulose Amination

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Keywords: Transaminase; Hemicellulose amination; Enzyme cascade; Enzyme engineering; Molecular dynamics simulation

Amine Transaminases (ATAs) are pyridoxal 5'-phosphate (PLP) dependent multimeric enzymes which catalyze the transfer of primary amino groups from amines to ketones or aldehydes. With carbohydrate oxidoreductases, ATAs can be used to aminate plant carbohydrates via two-step simultaneous cascades, where aminated cellulose and hemicelluloses are among desired products as basis of functionalized textiles and substitutes for animal-derived chitosan. Recently, we showed the one-pot amination of galactomannan using a galactose oxidase from *Fusarium graminearum* (FgrGaOx) and ATAs from *Chromobacterium violaceum* (CvATA) and *Silicibacter pomeroyi* (SpATA) [1]. Using a colorimetric assay developed in house, we measured a maximum of 10 % amination of galactose sidechains by SpATA. Using docking simulations, we identified a potential substrate binding cleft on SpATA surface and mutated four surface residues blocking the access of polymeric substrates to active sites. Despite the higher activity, CvATA produced a lower product yield due to its lower operational stability. We believe that operational stability is the main factor limiting the yield of aminated hemicelluloses produced by the enzyme cascades [1]. Deactivation of ATA is known to start from cofactor dissociation [2]. Using molecular dynamics simulations (MDS), we investigated the possible routes to cofactor dissociation and identified regions where cofactor-enzyme interactions can be improved. Analysis of sequences obtained from the ω-transaminase engineering database (oTAED) revealed conserved amino acid sets near the cofactor binding pocket [3]. In MDS, adopting a conserved set of hydrophilic amino acids in a mostly hydrophobic region of CvATA showed stabilizing effects on the cofactor-enzyme interactions. This data and computation guided approach of ATA engineering will likely generate variants with improved operational stability and suitable for sustainable hemicellulose amination.

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IN VITRO AND IN SILICO STUDIES FOR LACCASE DESIGN

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Keywords: Laccase; directed evolution; phylogeny; computational design; lignin

Laccases are oxidoreductases with the highest number of reported applications thanks to low requirements and oxidation versatility. These multicopper oxidases are back into the limelight as biocatalysts to valorize leftover lignins from the pulp and paper industry, with laccases secreted by ligninolytic basidiomycete fungi being the most interesting due to their high redox potential. However, natural enzymes often do not have the levels of expression, activity, efficiency or stability required for their application. Enzyme directed evolution has addressed these limitations with remarkable success in recent years to provide recombinant enzymes adapted to the industrial application conditions.

In enzyme directed evolution, the enzyme undergoes iterative rounds of diversification and screening under appropriate selective pressure to obtain improved variants in the property of interest by accumulating mutations in the sequence. The probability of discovering the most successful variants correlates with the breadth of sequence space explored, which is limited by experimental effort, consuming resources and time. Computational tools help protein engineers expand the exploration of the protein space through in silico bioprospecting of enzyme sequences in genomes and databases and in silico evolutionary studies, protein design through molecular dynamics and quantum mechanics models and the emergence of algorithms based on machine learning data that have revolutionized enzyme design.

We will be present some examples of genomic and phylogenetic studies combined with directed evolution studies that have allowed us to discover new types of enzymes and to resolve mechanisms of adaptation during evolution, as well as directed evolution and computational design of laccases to obtain tailor-made biocatalysts to revalorize biomass waste.

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Unveiling catalytic and structural properties of new bacterial carbohydrate oxidases

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Keywords: flavoenzymes; copper radical oxidases; substrate specificity; enzyme mechanisms

Carbohydrate oxidases represent a fascinating class of enzymes with diverse applications across various fields, from biotechnology to medicine. 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. [1] POxs are flavoenzymes that couple the oxidation of aldopyranose sugars, including D-glucose, at the C2 or C3 to generate keto-sugars. GalOx bearing to redox centers, a copper ion, and a Cys-Tyr free radical oxidize the hydroxyl group at the C6 position of D-galactose but show a wide range of specificities e.g. alcohols to their corresponding aldehydes.
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-oxidase [2] 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 that have a predominant role in its thermal stabilization and activity for D-Gal-containing polysaccharides, as assessed by affinity electrophoresis gels and ITC, among other approaches. The analysis of the X-ray structure solved at 1.2 Å, in conjunction with *in silico* studies, will help to unveiling molecular mechanisms governing its selectivity and stability. Directed evolution improved the catalytic performance for the canonical D-Gal substrate and other substrates, essential building blocks for important industrial bioproducts and biomaterials.

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Characterization of the activity of dye-decolorizing peroxidases on O-glycosides and lignocellulose

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Keywords: dye-decolorizing peroxidases, O-glycosides, lignocellulose, RDE-electrode, mass spectrometry

Plants have evolved to synthesise recalcitrant polymers in the cell wall for structural strength and protection against microbial invasion. Fungal laccases and lignin-active peroxidases are capable of degrading aromatic lignin in the lignocellulosic network. Additionally, fungi and bacteria contain dye-decolorizing peroxidases (DyP), which are also capable of oxidizing and modifying lignin constituents. Studying DyP activity on lignocellulose poses challenges due to the heterogeneity of the substrate and the lack of continuous kinetic methods. We present a method to measure kinetic parameters of the bacterial DyP from *Amycolatopsis* 75iv2 and the fungal DyP from *Auricularia auricula-judae* on insoluble plant substrates and kraft lignin by monitoring the depletion of the co-substrate of the peroxidases with a H_2O_2 sensor. Both enzymes show similar kinetics in reactions with spruce. On kraft lignin, the fungal DyP was nearly three times more active than the bacterial DyP. Real-time measurement of H_2O_2 allowed the assessment of continuous activity for both enzymes, revealing high turnover stabilities and remaining activities of 40% and 80%, respectively. Using mass-spectrometry, the depletion of the co-substrate H_2O_2 correlated with product formation.

Additionally, the enyzme from *Amycolatopsis* 75iv2 was tested on several *O*-glycosides, which are plant secondary metabolites with a phenyl glycosidic linkage. *O*-glycosides are of interest for studying the compounds themselves and as potential models for specific lignin-carbohydrate complexes. The primary DyP reaction products of salicin, arbutin, fraxin, naringin, rutin and gossypin were oxidatively coupled oligomers. A cleavage of the glycone moiety upon radical polymerisation was observed when using arbutin, fraxin, rutin and gossypin as substrates. The amount of released glucose from arbutin and fraxin reached 23% and 3% of the total substrate, respectively. The proposed mechanism suggests a de-stabilization of the ether linkage due to the localization of the radical in the para position. DyP2 was also tested on complex lignocellulosic materials such as wheat straw, spruce, willow, and purified water-soluble lignin fractions, but no remarkable changes in the carbohydrate profile were observed, despite obvious oxidative activity. The exact action of DyP2 on such lignin-carbohydrate complexes therefore remains elusive.

Bioconversion using single and co-cultures or natural and synthetic microbial consortia

Microbial processing of lignocellulosic polysaccharides in gut systems

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Keywords: gut microbiota, CAZymes, β-mannans, salmon, meta-omics

The community of microbes inhabiting the gastrointestinal tract of mammals and fish includes a large variety of bacterial species that collectively influence numerous aspects of host health and nutrition. Bacillota and Bacteroidota phyla are typically dominant, with specific symbiotic members supplying an arsenal of carbohydrate-active enzymes for the depolymerization and fermentation of otherwise indigestible complex carbohydrates to short-chain fatty acids [1]. In this talk I will cover some of our recent research, which utilizes methodological toolsets that combine traditional culturing, meta-omics (including next-generation sequencing and functional multi-omics), biochemistry and enzymology, to fully elucidate enzymatic pathways that microbial populations employ for the utilization of nutrients consumed by the host. This includes typical hemicellulosic polysaccharides, such as β-mannans. In particular, coupling of detailed knowledge of microbial saccharolytic mechanisms to unique structural features of β-mannans has allowed us to design intervention strategies to selectively engage beneficial microbes at genera/strain level. Additional application of multi-omics has enabled visualization of the impact of different lignocellulosic polysaccharides on the gut microbiota composition and functions, unveiling interactions between key players in degradation of this fiber directly in complex endogenous animal microbiomes and elucidating mechanisms by which these microorganisms may affect host biology [2, 3]. We demonstrate that a multi-faceted approach is needed for deciphering and implementing efforts to enhance host health and minimize disease by manipulating gut microbial composition and metabolism.

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How to handle lignin in absence of oxygen: a bacterial lesson?

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Keywords: anaerobic delignification; bacterium; RNAseq; cellulosomes; SGNH hydrolase

The industrial utilization of the plant biomass, earth's most abundant renewable raw material and a credible alternative to the fossil resource's utilization is hampered by the high structural intrinsic stability of lignocellulose. Fortunately, (ligno-)cellulolytic microbes have evolved performant systems to deconstruct this huge and recalcitrant reservoir of carbon we can build on to design sustainable applications. Contrary to the well-studied (hemi-)cellulolysis that is integrated in plant polysaccharide bioconversion-based processes (i.e. biofuels), delignification constitutes a major conundrum to solve since lignin precludes the access to the polysaccharides and its aromatic derivates inhibit cellulolytic enzymes and are toxic upon accumulation. Its microbial aerobic uncontrolled oxidation (via free-oxygen-radical reactions and metalloproteins as laccases and peroxidases) in compounds poorly usable in industry is well documented. In contrast, very little is known about how lignin is handled in an oxygen-free environment where plant material accumulates. Only the anaerobic fungal lignin deconstruction was proposed in 2023 but without identification of actors [1].

Before this recent publication, we hypothesized anaerobic bacteria are likely to have developed, as described for the cellulolysis, still unidentified and sophisticated ways to deal with lignin without the use of reactive oxygen to avoid the oxidative stress and consequently in a more specific and controlled manner. Then, applying an explorative *in vivo* approach by deep analysis of *Ruminiclostridium cellulolyticum* transcriptome evolution during growth on more or less lignified substrates (cellobiose to insoluble wheat straw) and correlation with the substrate composition evolution, we 1) showed that the "delignification" in the absence of oxygen occurs and 2) identified some of the potential actors. Biochemical characterization of their lignolytic activity, combined with a genetic approach, confirmed that two cellulosomal components are promising candidates for biotechnological applications that facilitate the access to the polysaccharides. These two enzymes are at least likely to efficiently cleave the ester bond connecting hemicelluloses and lignin, a function never described for these members of the large SGNH hydrolase superfamily.

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Lignin utilization by bacteria in an anerobic environment?

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Keywords: Lignin, Bacteria, Anaerobic, Metaomics, Enzyme discovery

Fungi are considered the major lignin decomposers in terrestrial ecosystems. However, in deeper mineral soil, where oxygen may be limited, bacteria are thought to play a more central role in lignin degradation [1]. A substantial amount of lignocellulosic biomass is found at considerable depth in the soil, and studies have shown that while fungi dominate in the upper 20 cm of the soil, the relative abundance of bacteria increases with depth [2]. While fungal lignin-degrading enzymes have been studied quite extensively, bacterial degradation of lignin is less explored, although this subject has attracted increasing attention in the past decade. Recent studies suggest that the role of bacteria in degradation of lignin has been underestimated [1, 3], as also suggested by the observation that several soil bacteria can catabolize lignin-derived aromatic model compounds [4].

In this study we have performed bacterial enrichments under denitrifying conditions, i.e., anoxic conditions using nitrate as electron acceptor, with various lignin preparations as sole carbon source, including brown-rotted wood. Growth was monitored by measuring production of nitrous gases and bacterial communities were analyzed using metagenomics and quantitative metaproteomics. We obtained small, yet specialized communities of up to 45 metagenome-assembled genomes (MAGs), with certain MAGs dominating various communities. Together with quantitative metaproteomics we identified thousands of proteins with diverse functions. This multi-omics analysis of bacterial communities enables us to determine which bacteria are present as well as their enzymatic capacities and metabolic activities, ultimately leading to the identification of keystone enzymes and enzyme systems that are used to utilize lignin as a carbon source.

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Fungal wood decay under anoxia

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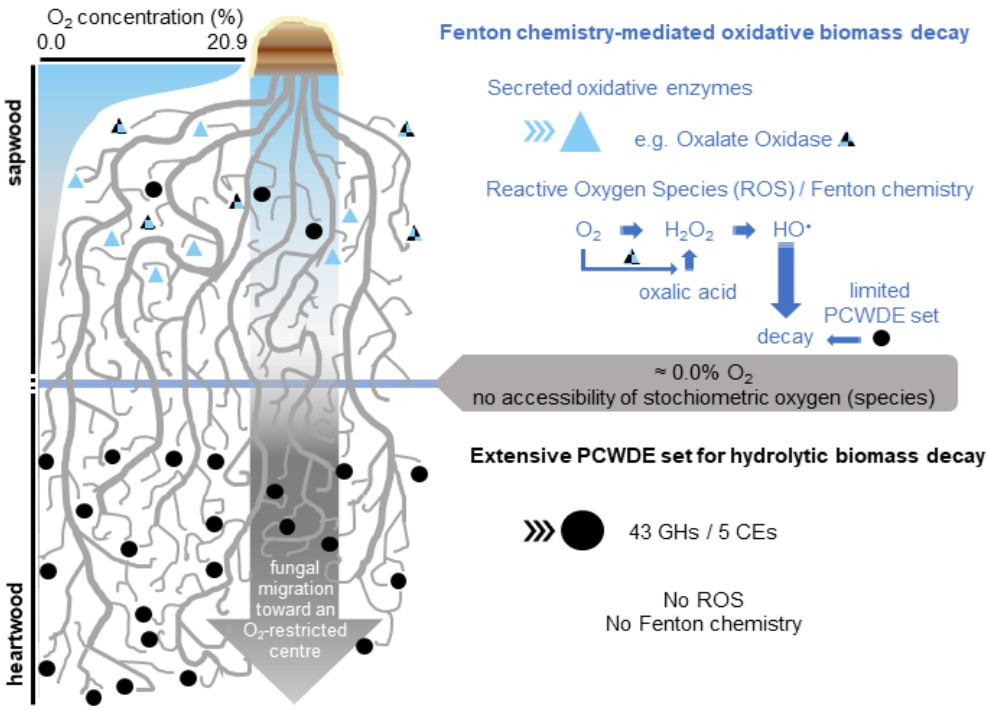
Keywords: Dioxygen Depletion; Natural Microbial Composition in Wood; CAZymes; Brown-rots; Fenton chemistry

Basidiomycete white-rot and brown-rot fungi are the main decomposers of dead wood[1] with impact on the global carbon cycle[2]. Their degradative mechanisms have been extensively studied mostly in an aerobic context with a strong focus on Fenton chemistry and oxidative enzymes[3], however, anaerobic wood degradation was unsuspected. Using metaproteomics on decaying softwood in a boreal forest, we identified wood-decomposing
fungi and their secreted enzymes at different depths from the wood surface, including in O2-depleted conditions. Using in vitro solid-state cultures, we demonstrate that the brown-rot fungus *Fomitopsis pinicola*, identified in the forest samples, can grow and decay wood under strict anoxic conditions with the secretion of a full set of glycoside hydrolases and carbohydrate esterases targeting softwood (hemi)cellulose and pectin components. These findings demonstrate the plasticity of the fungus to dwell in an exceptionally restrictive ecological niche, and explain the paradoxical presence of plant cell wall degrading en-zymes in the genome of brown-rots[1], which were previously described to degrade (hemi)cellulose using Fenton chemistry[3]. Our results extend the understanding of lignocellulose degradation mechanisms in nature and provide opportunities to advance decarbonization strategies for developing bio-inspired anaerobic processes.



Schematic summary illustrating the penetration of *Fomitopsis pinicola* into the wood towards anoxic conditions. The data presented in this study suggest a shift from Fenton-mediated wood decay under normoxia towards exclusive PCWDE-driven hydrolytic decomposition in anoxia. *F. pinicola* is represented by grey filaments, oxidative enzymes by light blue triangles, and hydrolytic CAZymes by black circles.

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Microbial strategies for degradation of spruce bark

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Keywords: bark, extractives, microbial consortia, resin acids, white-rot fungi

Tree bark is a hugely abundant renewable resource, produced in millions of tons per year in industry. It is however poorly used – most is burnt for energy, but the high moisture and mineral content of the bark makes this inefficient. The bark contains regular wood polymers but is not used in pulping because it also contains high proportion of so-called extractive compounds (extractives). The extractives, typically divided into hydrophilic and hydrophobic types, are highly diverse and differ in identity and proportions depending on the tree. Many extractives are regarded as strongly antimicrobial, and they are an important part of the tree's defense against attacks. In nature, the bark is still decomposed over time, though the process is virtually unstudied, and further knowledge could enable new valorization strategies of both its polysaccharides, extractives, and lignin fractions.

We have recently begun shedding light on how spruce bark can be decomposed by both microbial consortia and individual fungal species, by continuously mapping chemical changes to the carbohydrates, lignin, as well as the extractives over six months. For the microbial consortia, the changes in the microbial population were monitored, and resin acids were shown to exert a strong selective pressure while carbohydrate degradation was limited [1]. The main resin acid degrader was isolated and represents the new species *Pseudomonas abieticivorans*. For individual fungi, different strategies were observed, which ranged from selective degradation of certain polysaccharides and tolerance of resin acids, to broad degradation of all spruce bark components including extractives [2]. Currently, we are investigating the resin acid degradation pathways of *P. abieticivorans*, and its enzymes may be used for controlled modification and development of new forest-based materials.

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CHARACTERIZATION OF A NOVEL CELLULOSOMAL ENZYME REVEALS AN UNSUAL FOLD AMONG CINNAMOYL ESTERASES

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Keywords: esterase; cellulosome; SGNH hydrolase; hydroxycinnamic acids; lignocellulose

In a circular bioeconomy context, agricultural by-products are abundant sources of lignocellulosic biomass that can be valorized into value-added compounds. Nevertheless, commelinid monocots plants, such as cereals and grasses, are characterized by the presence of hydroxycinnamic acids (HCAs) substitutions on cell wall polymers. While these aromatic moieties can become a source of renewable phenolic compounds, their presence results in the formation of lignin-carbohydrate complexes (LCCs) [1]. LCCs protect polysaccharides from enzymatic deconstruction, thus increasing the challenges associated with lignocellulose valorization. Cinnamoyl esterases, described in the CAZy Carbohydrate Esterase 1 family (CE1), can tackle the esters bonds linking HCAs to hemicellulose. All cinnamoyl esterases characterized to date, along with CE5, CE7, CE15 and CE19 CAZy families, share the conserved α/β hydrolase fold [2]. Transcriptomic analysis was performed during growth of the anaerobic bacterium *Ruminiclostridium cellulolyticum* on wheat straw. Compared to non-lignified material, a large set of genes encoding for cellulosomal enzymes were found to be up-regulated. Herein, we investigated the function of the most up-regulated one, *Rc*1060, and found that it has cinnamoyl esterase activity with specificity towards HCAs involved in plant cell wall cross-linking. Structural characterization using X-Ray crystallography revealed that *Rc*1060 belongs to the SGNH hydrolase superfamily. The SGNH fold is shared across CAZy CE2, CE3, CE6, CE12, CE17 and CE20 families, but differs from the usual α/β hydrolase fold of currently characterized cinnamoyl esterases. *In silico* hydrophobicity analysis of the active site of the enzyme, suggests that it could face lignin and be directed towards the cleavage of HCAs mediated lignin-carbohydate cross-links, and eventually HCA esterified lignin. As such, this newly characterized SGNH hydrolase and related ones, might represent accessory enzymatic tools to reduce biomass recalcitrance and develop novel biotechnological applications.

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Characterization of lignin degradation by specialized bacterial consortia derived from termite gut

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Keywords: Bacterial consortia; Delignification; Microbial consortia engineering; Termite gut microbiota

While the majority of biotechnological processes only involve pure strain cultures on homogeneous substrate, in natural environments, the complex process of lignocellulose degradation is carried out by the synergistic action of complex microbial consortia. Mastering the culture of these complex consortia is an important prerequisite for the efficient conversion of substrates such as lignin to added-value molecules through biotechnological processes.

The present work characterizes the lignolytic capacity of two bacterial consortia originating from the gut microbiome of the termite *Nasutitermes ephratae*. The selected substrate GV03-FB01 is the ethyl-acetate soluble fraction of an industrial lignin, containing a mixture of monomeric (phenolic acids, ketones and aldehydes) and various oligomeric compounds with recalcitrant linkages (β-β, β-1, β-5) [1]. Experiments were inoculated with i) NE15 consortium, selected on a lignin-rich wheat straw as sole source of carbon, or ii) directly with the termite gut microbiome [2]. After enrichment by aerobic sequential batch cultivation using GV03-FB01 as sole carbon source (cycles of 10-13 day, 30°C, 150 rpm), we obtained the enriched consortia NE15-GV03 and GUT-GV03. HPLC analysis of the residual substrate showed that most of the monomeric compounds present in GV03-FB01 were degraded by both consortia after 4 days of incubation. In contrast, the oligomeric fraction of the substrate was modified by the consortium NE15-GV03 only. This last also showed a more important biomass growth compared to the GUT-GV03 consortium. Our results suggest that the lignin-rich wheat straw enrichment of *Nasutitermes ephratae* gut microbiota has selected bacterial taxa with enzymatic capacities to degrade relatively complex lignin structures and metabolizing them for cellular growth.

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Development of an agricultural-residue-based medium for cultivating anaerobic fungi

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Keywords: anaerobic fungi; natural microbial consortia; bioconversion; biogas; lignocellulose

Anaerobic fungi (AF, phylum Neocallimastigomycota) are key players in the digestion of lignocellulose in the digestive tracts of some herbivorous animals. As initial colonizers of ingested fibers, AF disintegrate lignocellulose and make it accessible for subsequent microbial degradation [1, 2]. Their distinctive ability to degrade recalcitrant matter through both enzymatic and mechanical mechanisms [1] makes them suitable candidates for anaerobic pretreatment of lignocellulose-rich substrates, such as agricultural residues for biogas production. At present, the effortful conditions required for their cultivation, e.g., rumen fluid-based media, prevent their application in biotechnology. Thus, the development of a simple, non-hazardous culture medium that supports the continuous growth of anaerobic fungi *in situ* at agricultural facilities, such as biogas plants, would be a critical first step for their successful implementation. The growth of a *Neocallimastix frontalis* isolate growing in syntrophy with associated methanogenic archaea was monitored indirectly by gas pressure measurements on different types of culture media over a period of 11 days in a batch process. *N. frontalis* exhibits stable growth in a rumen fluid -free medium containing 0.5% (w/v) lignocellulose-rich wheat straw. Furthermore, the viability of AF was demonstrated in a minimal medium without the addition of yeast extract, tryptone, salt solutions or trace elements, containing wheat straw as the sole carbon source and the liquid portion of the pressed digestate as a byproduct from biogas plants for mineral supply. The byproduct was added to the minimal medium in increasing concentrations, and the growth behavior of *N. frontalis* was determined. Based on the current results, we conclude that the growth response is positively correlated to the addition of the byproduct. Further growth experiments involving different AF genera will be conducted to validate the medium's suitability for biotechnological applications.

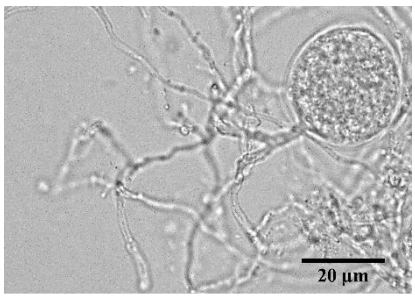

Figure 1: Microscopic image of N. frontalis.

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Circular bioeconomy: valorizing lignocellulose residues and its lignin fraction

Thoughts on the development of biomanufacturing and prospects for the future

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Keywords: biotechnology, biorefining, biomanufacturing, decentralisation

The noun biorefinery, and the associated gerund biorefining, first began to appear in scientific literature in the 1980s. This was a result of oil crisis provoked by the Iran-Iraq war. However, until first decade of the present century, these terms remained relatively unused.

The term biorefinery has defined many times and in many ways, but according to the IEA task 42 definition it is the "the sustainable processing of biomass into a spectrum of marketable products and energy". Although this definition is rather simple it includes key notions related to sustainability and multiproduct outputs.

In my presentation I will provide a personal view of how biorefineries have developed so far, underlining the limits of the use of the original oil refinery paradigm. I will also consider future prospects, focusing on those that can be driven by biotechnology and biomanufacturing. In this regard, I will provide a brief appraisal of the European situation and make proposals on how to move biotechnology forward, especially at the preindustrial R&D level.

Multi-steps biocatalytic strategy to produce a portfolio of biomolecules of interest from lignocellulosic biomass

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Keywords: biocatalysis, glycosides, sugar esters, polyfunctional biomolecules, agroindustrial co-products

With the double aim to produce biomolecules of interest from lignocellulosic biomass and to develop environmentally-friendly and selective processes, we study the development of multi-enzymatic strategy through hydrolysis, glycosylation or acylation reactions catalyzed by glycoside hydrolases as well as lipases [1-2].

In a recent approach, we synthesized sugar esters and alkyl glucosides, two non-ionic biosurfactants of interest for cosmetic and food applications. First, we developed a transglycosylation strategy with xylanases in order to produce alkyl xylosides directly from wheat bran. In parallel, the synthesis of D-glucose esters was investigated with a first hydrolysis step conducted with cellulases to recover D-glucose from wheat bran. Dglucose was then acylated by fatty acids with a lipase to obtain glucose-based esters. Finally, we developed an integrated-process that allowed to produce both alkyl xylosides and D-glucose esters directly from a unique wheat bran batch without any pretreatments with two enzymatic steps [3-4].

In a current study, we apply this multi-enzymatic strategy to synthesize polyfunctional biomolecules displaying properties of interest for cosmetic purposes from building-blocks coming from plant biomass biorefining.

The main results of our multi-biocatalytic strategy will be presented.

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Miscanthus sinensis conversion by the white-rot fungus *Ceriporiopsis subvermispora*: Plant genotype - fungal strain harmonization for total-use cascading into constituent biopolymers

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Keywords: lignocellulose, lignin, fungi, chemical analysis, biopolymer characterization

The white-rot fungus *Ceriporiopsis subvermispora* has previously been recognized for its ability to effectively and selectively delignify lignocellulose, and hence considered for biotechnological potential. We raised the question whether improvement of the process would be possible through selecting specific combinations of plant genotypes and fungal strains, and as such bring industrial implementation closer to reality. To tackle this question, we treated four *Miscanthus sinensis* genotypes with four *C. subvermispora* strains and thoroughly analyzed substrates and treated residues.[1] The *M. sinensis* genotypes differed in cellulose, hemicellulose and lignin contents and diverged in lignin structure in terms of subunit and linkage composition and abundance of hydroxycinnamic acids and tricin. Independently of the *M. sinensis* genotype used, *C. subvermispora* strain MES13904 outperformed the other three strains in extent and selectivity of delignification and consistently generated the highest enzymatic residual carbohydrate conversion and structural changes in the residual lignin. The 'best' substrate-fungus combination gave 63% w/w delignification and an excellent total enzymatic glucose yield of 66% w/w. Next, this harmonized substrate-fungus combination was upscaled and further evaluated in a sequential alkaline total-use biorefinery cascade aiming to fractionate *M. sinensis* into its cellulose, hemicellulose and lignin constituents. Here, we paid critical attention to mass balancing, purity and structural features of the biopolymers obtained, all compared to the untreated substrate analogue. We demonstrate that fungal treatment prior to alkaline fractionation improves cellulose purity, but that this comes at the expense of recovery, degree of polymerization and crystallinity. Properties and structural differences of the treated hemicellulose and lignin fractions call for further exploring the potential of the biorefinery incentive presented.

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The Meliora Bio biorefinery: Production of dietary fiber and ethanol from wheat straw

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Keywords: pretreatment, enzymatic saccharification, arabinoxylan, abiotic oxidation

Meliora Bio is a modern state of the art biorefinery upcycling straw for bioethanol, prebiotics, and lignin. The feedstock is locally grown wheat straw which is pretreated through the recently installed Bio Trac unit from Valmet. The soluble fraction containing monosaccharides and arabinoxylan is used for the production of dietary fibres at the co-located CometBio facility. The insoluble fraction is first enzymatically liquified in two parallel horizontal stirred reactors (fig. 1A). The slurry is then pumped to a second set of reactors for full enzymatic saccharification before separate fermentation. The effect of abiotic oxidative processes during enzymatic saccharification has been described based on laboratory studies [1]. The importance of controlling the dissolved oxygen for optimised enzyme performance has been demonstrated and importantly, the effect of both lytic polysaccharide monooxygenase and catalase has been shown [2]. However, relevance of these lab-results in the industrial setting is lacking. We have initiated such analysis and find that the abiotic oxygen consumption in the liquified slurry is fast (fig. 1B). The implications will be discussed.

Figure 1: Photo from the saccharification section at Meliora Bio and graph showing abiotic oxygen consumption at 50°C in a sample of the liquified wheat straw slurry taken during production._

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Sustainable processes: LCA for lignocellulose bioconversion

Environmental performance of lignocellulosic biomass valorization: What did we learn from LCAs and what are the research challenges ahead?"

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Keywords: bioeconomy, life cycle assessment, residual biomass

Facing the reality of the climate urgency, many countries are aiming today to peak their greenhouse gas (GHG) emissions and reach neutrality in a medium- to long-term future. This implies not only technologies and solutions to decarbonize or mitigate GHG, but also negative emission technologies to permanently remove GHG from the atmosphere, and technologies to substitute fossil carbon by renewable carbon. Yet, in which technology to invest, when and where, in a given country or territory, to ensure these climate goals can be reached sustainably, i.e. not at the expense of dramatically increasing other emissions like phosphorus losses or nitrous oxides affecting other environmental impacts, or long-term socio-economic performances?

In the French Make Our Planet Great Again Cambioscop project, we investigated precisely this, taking as a starting point the amount of residual biomass available in the French territory. Several combinations of biomass-conversion pathways were investigated, for applications in sectors spanning from maritime and aviation transport, ingredients for food and feed or bio-based products. Their environmental performance were quantified through Life Cycle Assessment (LCA). Here, the results of these LCA will be presented, with a focus on the key parameters shaping the dependency of the environmental performance of these pathways.

Poster abstracts

Detailed characterization of hardwood and softwood lignin conversion by a brown-rot basidiomycete

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Keywords: Bioconversion, lignin, brown-rot, py-GC-MS, NMR spectroscopy

Wood-degrading brown-rot fungi primarily target carbohydrates, while lignin becomes partially modified and of potential interest for targeted lignin valorization[1]. Here, we report a comprehensive comparison of lignin conversion by a brown-rot basidiomycete, *Gloeophyllum trabeum*, growing on a hardwood and a softwood substrate. By harnessing the latest advancements in analytical methodologies, we show that *G. trabeum* not only degrades polysaccharides efficiently and selectively but may also remove more lignin from wood than previously reported. Structure-wise, brown-rotted lignin appeared substantially C_α-oxidized, O-demethylated, depleted in interunit linkages, and enriched in diagnostic substructures indicative of C_α-C_β, β-O and O-4 bond cleavages in the β-O-4 aryl ether linkage. These findings enhance our understanding of lignin conversion by brown-rot fungi, revealing previously unknown aspects of this process. Specifically, despite the well-documented differences in lignin structure between hardwood and softwood[2], *G. trabeum* attacks the same bonds in the lignin structures, resulting in similar chemical modifications regardless of the wood substrate. Furthermore, we show that *G. trabeum* enhances the antioxidant capacity of the lignin, and that the residual lignin can be separated into low- and high-molecular weight fractions with distinct properties. This highlights the biotechnological potential of brown-rot fungi for developing lignin-based antioxidant or resin products.

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IMPACT OF FUNGAL LACCASE AND MEDIATORS ON LIGNOCELLULOSIC BIOMASS PROPERTIES AND STRUCTURE DURING SACCHARIFICATION

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Keywords: fungal laccase, mediators, lignocellulosic biomass, recalcitrance, saccharification,

Humanity faces an unprecedented challenge as earth is subjected to increasing climate changes, mainly caused by human activities. This situation is worsening as our energy needs continue to grow. In this context, renewable energy resources and bio-based products have emerged as indispensable solutions to replace fossil carbon. These renewable resources include plant biomass, which covers a wide range of carbon sink, notably lignocellulosic biomass (LCB) located within the cell walls of plants. LCB primarily consists of three polymer types: cellulose and hemicelluloses, both comprising sugar units, and lignin, a complex phenolic polymer. Cellulose can be converted into ethanol after saccharification and fermentation. Nonetheless, the intricate nature of the structural polymers constituting LCB poses a challenge to their efficient conversion into biofuels and encourage manufacturers to use chemically polluting and energy-intensive techniques. The challenge of LCB recalcitrance to saccharification is primarily attributable to lignin, which restricts sugar monomer conversion from complex polysaccharides and renders lignocellulosic biofuels economically disadvantaged compared to petroleum-derived alternatives[1]. Despite the availability of highly effective commercial enzyme cocktails for polysaccharide degradation, they are unable to adequately break down the lignin fraction during enzymatic saccharification. Facing this issue, laccase-based enzymatic pretreatments targeting lignin have already been used to reduce the recalcitrance of LCB and thus improve saccharification yields[2]. The objective of this work is to develop new biomass treatment protocols that integrate and improve these laccase/mediator systems, and to use them to optimize saccharification with commercial enzyme cocktails. Tests were carried out on wood powder from poplar, a promising tree species dedicated to biofuel production. In this regard, we tested different concentrations and experimental conditions to achieve optimized saccharification yield. This latter was mainly evaluated by analyzing the glucose concentration in the degradation supernatants, and by observing chemical modifications in residual biomass with thioacidolysis, FTIR and NMR analysis. The results showed up to 35% increase in sugar accessibility when LCB was pretreated with our improved laccase/mediator systems prior to polysaccharide degradation with a commercial enzyme cocktail.

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Chemical and enzymatic reactive extrusions to improve biomethane production from corn stover

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Keywords: anaerobic digestion ; pretreatment; reactive extrusion ; biomethane ; biodegradability

The performance of the anaerobic digestion process of lignocellulosic biomasses is generally affected by their physicochemical properties [1]. To facilitate accessibility to the lignocellulosic structure by microorganisms, several types of pretreatments have been studied in the literature, in particular mechanical, chemical, and biological processes, or their combination [2]. Among the mechanical pretreatments, twin-screw extrusion is a mature process already deployed at industrial scale, allowing the reduction of particle size through mixing and shearing unit operations, thus improving kinetics of biomethane production [3]. However, extrusion has understudied potential for further improvement by its combination with either chemical (i.e., reactive extrusion) or enzymatic (i.e., bioextrusion) pretreatments, to enhance methane potential [4]. In this context, the objective of this study was firstly to evaluate the effect of twin-screw extrusion followed by alkaline pretreatment on the physicochemical composition of the selected agricultural by-product and anaerobic digestion performance (i.e., kinetics of biomethane production and methanogenic power) at the moment of BMP tests [5]. Twin-screw extrusion pretreatment was applied on corn stover, using a screw profile with reverse bilobal elements on a pilot-scale extrusion machine. Lime was then impregnated after extrusion with 10g $Ca(OH)_2$/100g DM at a final dry matter (DM) content of 30% (w/w). No significant improvement in biomethane production was induced by the sole twin-screw extrusion of corn stover, but an improvement in methanogenic potential of 33% was observed after lime impregnation. An improvement in kinetics of 109% and 136% was observed, respectively, after extrusion and after lime impregnation). These improvements are linked to particle size reduction regarding extrusion (from particle size over 40 mm to under 2 mm), and lignin depolymerization (8%) regarding lime impregnation. Consequently, biodegradability [6] was improved by 9% for extruded samples, and 14% for the lime impregnated ones, leaving 26 up to 31% of biodegradability range for further improvement through an additional enzymatic pretreatment. Bioextrusion was then secondly performed on a small laboratory extruder equipped with only mixing elements, adding 53 total enzymatic units/g DM of a cellulolytic, hemicellulolytic and lignolytic commercial enzymatic cocktail on either previously extruded or lime impregnated extruded samples. Encouraging preliminary results seems to indicate further improvements in methanogenic potentials, kinetics, and biodegradability after bioextrusion.

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[6] Biodegradability is expressed as experimental BMP divided by theoretical BMP the latter being calculated using the Buswell equation [Achinas and Euverink, 2016]

Bio-electrorefinery of lignins

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Keywords: lignin, bioelectrochemistry, electrocatalysis

Lignins are considered to be a "bottomless" bioresource of organic aromatic compounds. Their structural complexity and heterogeneity that underlies lignocellulose recalcitrance, limit their valorization in biorefineries; consequently, lignins remain one of the most underutilized renewable resources on Earth. Consequently, a sustainable lignin degradation is desired to benefit from both environmental and economical perspectives. In nature, white-rot fungi and certain species of bacteria use an enzymatic arsenal to depolymerize and refine lignins. Biological lignin degradation involves the cleavage of β-aryl ether and C-C bonds catalyzed by oxidoreductases, such as high redox potential peroxidases. These hydrogen peroxide dependent heme-containing enzymes are able to catalyze lignin depolymerization through the oxidation of phenolic and non-phenolic part of lignins generating heterogeneous mixture of aromatic oligomers.[1] On the other hand, certain ligninolytic microorganisms are able to refine these "soup" of aromatic oligomers using intracellular enzymatic systems acting like a metabolic funnel, such as the etherolytic pathway from the soil probacterium *Sphingobium paucimobilis*.[2] This microorganisms possess catabolic pathways involving so-called non-radical ligninolytic enzymes, such as etherases and NAD-dependant dehydrogenases catalyzing the specific cleavage of β-O-4 ether aryl bounds representing up to 70% of the linkages between lignin monomers. [3]

We explored the possibility of combining this enzymatic machinery with electrochemical technologies to develop bioelectrodes with hybrid catalytic interfaces, consisting of the immobilization of electrocatalysts and ligninolytic enzymes working in synergy. The ligninolytic potential of these bioelectrodes was evaluated using lignin dimer models and technical lignins. This study provides valuable insights into enzymatic lignin degradation pathways and highlights the potential of hybrid electrocatalytic approaches for lignin valorization.

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Pretreatment of vine roots and valorization of post-extraction residues by way of an innovative mobile pelletizing technology

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Keywords: pretreatment, granulation, cascade valorization, scale-up, mobility

Collection and pretreatment of lignocellulosic biomass are the crucial initial stages of all valorization chains. It is essential for the input material to be stable and standardized to ensure consistent parameters and optimal process performance at large scale. However, lignocellulosic biomass presents challenges due to its highly heterogeneous and variable composition, high recalcitrance and tendency for spontaneous degradation. These factors make it a difficult resource to manage. This thesis aims to develop a pretreatment method of vine roots for polyphenols extraction using a mobile pelletizing unit. The proposed technology involves four key steps: grinding, drying, additivation and densifying. The mobile granulation process, in particular, offers several benefits:

- Homogenizes and fractionates the material, increasing the specific surface area of the particles and the extraction interface
- Removes moisture and prevents microbiological contamination early in the chain
- Densifies the biomass directly at source, reducing its volume and consequently lowering transportation costs and carbon footprint

Additionally, the thesis seeks to valorize post-extraction residues by transforming them into advanced fertilizers in pellet form, achieved by combining them with mineral residues and beneficial microorganisms.

The biomasses studied in this research were Pinot noir 792 vine roots and rootstock SO4. The latter were collected in representative batches of 250 kg. These were then processed using a FRITSCH P19 cutting mill and sieved to achieve a particle size distribution between 4 mm and 200 µm. The batches were dried using various technologies, including hot air flow, fluidized bed drying and lyophilization. Different temperatures and drying speeds were employed to achieve a final moisture content of 10-15%. After drying, the biomass was compressed using the SMARTWOOD PLT100 pellet mill with varying compression rates, utilizing a 6 mm flat die. The resulting pellets were subjected to extraction by a reflux method, using a solvent mixture of 60/40 EtOH/H_2O at 60°C for 45 min. The extracts obtained from this process were analyzed for Total Phenolic Content (TPC) and DPPH radical scavenging activity.

Throughout the thesis, the influence of pelletizing parameters—namely grinding, drying, and compression—on the biomass structure and extraction efficiency of polyphenols was thoroughly investigated. The underlying mechanisms involved in these processes were studied at a molecular scale, focusing on mechanochemistry, evaporation phenomena and friction forces. Regarding the development of pellet fertilizers, various assays were conducted to control and optimize key properties such as air porosity, water-holding capacity, nutrient release rate, and the carbon-to-nitrogen (C/N) and nitrogen-phosphorus-potassium (N/P/K) ratios. These tests aimed to enhance the efficacy and sustainability of the bio-based fertilizers produced from the lignocellulosic residues.

Scrutinizing biodiversity of lignocellulose-degrading filamentous fungi: extraction and analyses of critical morpho-granulometric parameters

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Keywords: Lignocellulose biomass; filamentous fungi; morphology; morphogranulometry

Lignocellulose biomass is a promising bioresource providing a stable and versatile source of materials for a wide range of application including: renewable energy production, food manufactory, biomaterial production. In order to degrade lignocellulose, filamentous fungi are considered as the most effective biofactory due to their enzyme diversity and high activities, renewable and low-cost advantages. Enzyme production by filamentous fungi performed under submerged condition is associated with important morphological evolution. Controlling fungi complex morphological structure is believed to be one of the main challenges in biotechnology industry. These morphologies directly affect the medium rheology since free mycelia increases culture viscosity and generates non-Newtonian behavior. Compact pellet formation is considered to facilitate biomass separation and secretome extraction in down-stream process; however, the main drawback is mass transfer limitation to the core region leading to cell degradation. The different states of filamentous fungi have a large impact on production yield, maintaining an appropriate morphology for targeted metabolites. In this research, an optical method was developed to characterize the morphology of different filamentous fungi strains by statistical analysis of associated criteria such as: dimension and distribution in submerged culture. Morphological characterizations were performed *ex-situ* using a morphogranulometer (Mastersizer G3S, Malvern Instruments Ltd., software Morphologi v7.21) including standardized microscopic observations, image analysis and statistical treatment of extracted criteria. Three representative filamentous fungi strains (*Talaromyces*, *Aspergillus niger* and *Trichoderma virens)* were selected due to their lignocellulosic biomass degrading capability from the fungal collection provided by FIRI and HUST [1]. Pellet density, shape and size, fiber parameters and associated distribution functions were correlated to biomass in order to describe the diversity of filamentous fungi morphology at different growth stages. Results indicate that under controlled submerged conditions, the three selected strains exhibit different pellets structures – from dispersed mycelia to compact pellet – and with large morphology variations. This first step in understanding filamentous morphology will contribute to correlate morphology with medium rheological properties and to rationalize process up-scale.

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A GTP-driven central carbon metabolism in the cellulolytic bacterium *Ruminiclostridium cellulolyticum*

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Keywords: GTP; cellulolysis; bacterium; central carbon metabolism; NTP-flexibility

Former studies have shown that in *Ruminiclostridium cellulolyticum* the hexokinase and the galactokinase strongly prefer GTP to ATP[1], whereas the phosphofructose kinase is a PPi-dependent enzyme[2], suggesting an unconventional central carbon metabolism in this anaerobic bacterium. In the present report, we have characterized all other kinases involved in this pivotal pathway, and determined their favorite NTP/NDP. It appears that the kinases involved in the first steps and acting essentially as NTP-consuming enzymes (the galactokinase, the hexokinase and the xylulokinase) are GTP-dependent enzymes. In contrast, the kinases that mainly generate NTP and which are involved in the downstream steps, the phosphoglycerate, pyruvate and acetate kinases display no marked preference for ADP or GDP. Consequently, the central carbon metabolism in *R. cellulolyticum* appears essentially GTP-driven. Nevertheless, it displays flexibility since the replacement of the GTP-dependent hexokinase in *R. cellulolyticum* by the ATP-dependent glucokinase from *Escherichia coli* generated a modified strain that can utilize glucose and cellobiose, whereas the opposite approach also engendered an *E. coli* strain that metabolizes glucose. Altogether, our data suggest an unexpected diversity in the functioning of the central carbon metabolism in bacteria, and offer new perspectives in the field of metabolic engineering.

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ROBUST INDUSTRIAL BIOCATALYSTS WITH PEROXYGENASE, PHENOL-OXIDASE OR FURFURYL-OXIDASE ACTIVITIES FROM BACTERIAL AND FUNGAL HOSTS

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Keywords: Oxidoreductases; Lignin; Lipids; Sugars; Bio-based chemicals & materials

Industrial Biotechnology is key to maintaining Europe's leading position in the bio-based market and boosting its transition towards a green and circular economy. This requires the development of innovative technologies such as those proposed in the ROBUSTOO EU project, coordinated by the Spanish Research Council, CSIC.

The aim of ROBUSTOO is to demonstrate the applicability of three families of oxidative enzymes (non-specific peroxygenases, laccases and hydroxymethylfurfural oxidases) in the innovative and sustainable production of bio-based chemicals and materials. The potential of these enzymes as industrial catalysts has been shown in previous projects of the consortium. ROBUSTOO will address the development of more robust enzymes better adapted to industrial conditions, as well as their production at pilot scale.

Activities will start with sequence bioprospecting and computational enzyme design (by BSC). Simultaneously, work will be carried out on the development of microbial strains for the industrial production of enzymes and their variants obtained by protein engineering, and then the optimisation and demonstration at pilot scale of their application in selected enzyme transformations, all of which will be carried out by several biotech SMEs (MetGen, Gecco, bisy, and InnoSyn) and research/technology centres (CIB and IRNAS of CSIC, Autonomous University of Barcelona and FCBA institute). The work will conclude with the techno-economic and environmental evaluation of the technologies developed, and the elaboration of commercial exploitation plans (Italbiotec).

The new biotransformations to be demonstrated at ROBUSTOO represent cutting-edge biotechnological solutions for: (i) the conversion of industrial lignins into bio-based material components, increasing the commercial value of available lignin by-products; (ii) the production of chemicals difficult to achieve by chemical synthesis, through regio/stereo-selective enzymatic oxygenations of lipophilic substrates; and (iii) the synthesis of sugar-derived plastic components, as a sustainable alternative to chemical catalysis and the use of petroleum-derived precursors.

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Screen-printed electrodes for the characterization of lignocellulosic biomass degrading enzymes: ligninases

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Keywords: Lignins; Ligninolytic enzymes; Paper-based electrodes; Catalase-peroxidase; Depolymerization; Screening.

Oxidative enzymes are a class of biocatalysts that catalyzes the oxidation of their substrate by electron transfers. Some of them are active on lignocellulosic biomass and could be used in biorefineries. For example, lytic polysaccharide monooxygenases (LPMO) can depolymerize crystalline cellulose while lignolytic enzymes can convert lignin into bio-based phenolic compounds [1], [2].

Despite they offer the advantages to be bio-based, specific and active under mild conditions, enzymes may be sensitive to pH, temperature and inhibitors, hindering their integration in industrial processes. One solution is the modification and/or discovery new biocatalysts together with the development of methods to measure the enzyme activity under industrial conditions.

Methods based on liquid chromatography analysis are usually employed for the discovery and the characterization of depolymerizing activities. However, these methodologies are cumbersome and therefore not suitable for screening strategies. This technological lock hinders the knowledge and consequently the development of biocatalysts efficient on solid substrate. For these reasons, an efficient method for high-throughput measurement of enzymatic activities is of interest. Electrochemistry represents an alternative method suitable for the measurement of electron transfers occuring in enzymatic reactions. Since several years, we have been developing 96-well electrochemical methods dedicated to highthroughput screening [3].

In this work we describe the use of screen-printed electrodes for the characterization of oxidases involved in the degradation of lignocellulosic biomass[4], [5].

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Valorization of Miscanthus biomass from metal-contaminated lands

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Keywords: Miscanthus, biomass, pyrolysis, bio-based products, circular economy

Soil pollution with metal contaminants is a serious environmental concern. The decontamination of these sites by phytoremediation is an economic and eco-friendly remediation method. In phytoremediation, plants are used to improve the quality of contaminated land by removing toxic, organic and metal trace elements from soil. Purpose-grown biomass such as Miscanthus is in particular effective in metal recovery from lands used for industrial activities, sites for mine exploitation or post-military sites [1, 2].

The objective of this study is to investigate the use of Miscanthus biomass, loaded with metals and collected from phytoremediation activities as a potential feedstock for bio-based products. Pyrolysis, a thermochemical process is used to convert the contaminated biomass into an intermediate product (bio-oil) that can be further transformed into biofuels, additives, or replacements of petroleum-based products [3,4]. Based on the characteristics of pyrolysis bio-oil, suitable pathways for valorization are evaluated. The impact of metal contaminants and their concentration on the quality of bio-oil is evaluated. Bio-oil upgrading to fuels and chemicals is discussed, together with process design of selected pathways.

This study demonstrates how biomass used for decontamination purposes can be converted to bio-based products and support the circular economy.

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Bacterial SGNH hydrolases assist the anaerobic plant biomass degradation: a new role linked to the lignin?

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Keywords: anaerobic delignification; bacterium; genetics; cellulosomes; SGNH hydrolase

Ruminiclostridium cellulolyticum is a Gram-positive and (hemi-)cellulolytic anaerobe widely studied for its ability 1/ to efficiently degrade both cellulose and hemicellulose from the vegetal biomass through the production of cellulosomes, and 2/ to use the degradation products for growth. As every microorganism degrading the plant biomass, it faces the obstacle of the lignin, a very stable aromatic polymer surrounding and precluding the access to the plant cell wall polysaccharides. Contrary to (hemi-)cellulolytic aerobes, which degrade lignin using free-oxygen-radical reactions and metalloproteins (i.e. laccases and peroxidases), very little is known about the anaerobic processes. However, our recent study shows that this model bacterium is at least able to modify the lignin during growth on lignified substrates as recently described for anaerobic fungi [1]. Moreover, we were also able to identify enzymes likely implicated in the extracellular handling of the lignin and we show that two secreted cellulosomal proteins, encoded by genes at loci Ccel_1060 and Ccel_2017, play a pivotal role by acting on methyl esterified derivates of lignin aromatics. We propose new roles for these members of the wide SGNH family, such as the cleavage of the ester bonds intra-lignin and/or between hemicelluloses and lignin to facilitate the access to polysaccharides by relaxing the plant cell wall structure.

Besides investigating the other potential bacterial actors of the anaerobic delignification, I then aim to understand the role of $SGNH_{1060}$ and $SGNH_{2017}$ *in vivo* by examining the effects of the inactivation or overexpression of their encoding gene during cultures on more or less lignified substrates. My on-going work (presented here) demonstrates the potential functional redundancy between the two *R. cellulolyticum* cellulosomal SGNH, and tends to confirm the role of $SGNH_{2017}$. Indeed, its overproduction is associated with an increased release of lignin derivatives into the medium and a metabolic reprogramming of the modified strain which produces formate exclusively when a lignified substrate is the carbon source. Therefore, I am currently investigating if the observed metabolic rerouting in the engineered strain of *R. cellulolyticum* results from improved access to the polysaccharides due to $SGNH_{2017}$ overproduction or a direct metabolism of the lignin byproducts.

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Exploring bacterial resin acid biodegradation

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Keywords: bark, resin acids, *Pseudomonas*, spruce

Tree bark is a massive but underutilized natural resource that is produced in hundreds of millions of tons per year but remains mainly a side stream that is usually burnt for energy generation, due to its relatively high content of extractive compounds that make it undesirable for regular pulping. However, burning is not efficient given the bark's generally high moisture and mineral content that leads to large amounts of ash. The bark is also the first line of defense for the tree against biotic and abiotic stress.

Bark biodegradation is a phenomenon that happens in nature, but virtually nothing is known about it – what happens to the material, which microorganisms are involved, and which enzymes do they use? In a recent study, we mapped spruce bark degradation over time by microbial consortia, both chemically and biologically [1]. The study revealed key stages during spruce bark degradation and especially resin acids were attributed to control the diversity of the microbial community. Resin acids are an important group of compounds within the spruce extractives, as insoluble, recalcitrant, and toxic molecules that serve as antimicrobial agents that protect the tree against pathogens. We isolated the dominant resin acid-metabolizing bacterium from the consortium which represents the new species *Pseudomonas abieticivorans*. It encodes a complete so-called *dit* gene cluster, which is suggested to encode the proteins involved in the initial degradation of abietane-type resin acids. Only a few studies have investigated resin acid metabolism [2], but only one enzyme has been heterologously produced and biochemically characterized. Currently, we are studying the enzymes hypothesized to govern the first steps of abietane-type resin acid degradation from the *dit* gene cluster of *P. abieticivorans*, including cytochrome P450 monooxygenases and a dioxygenase with its associated ferredoxin. This work adds new knowledge on microbial resin acid metabolism and will open an opportunity to better valorize bark extractive fractions, to rationally modify resin acids into new materials or novel chemicals with pharmaceutical properties.

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Mimicking the enzymatic plant cell wall hydrolysis machinery for the degradation of polyethylene terephthalate

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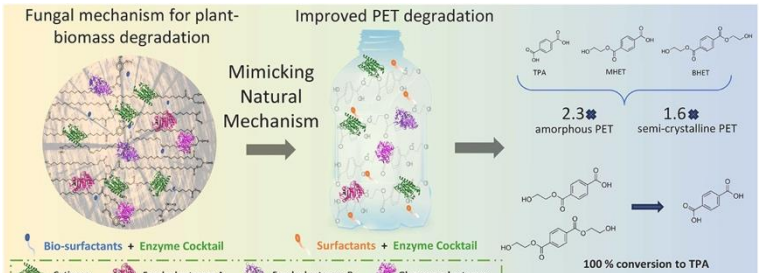
Keywords: fungal enzymes; cutinase; feruloyl esterase; glucuronoyl esterase

Plastic pollution presents a global challenge, impacting ecosystems, wildlife, and economies. Polyethylene terephthalate (PET), widely used in products like bottles, significantly contributes to this issue due to poor waste collection. In recent years, there has been increasing interest in plant biomass-degrading enzymes for plastic breakdown, due to the structural and physicochemical similarities between natural and synthetic polymers [1]. Filamentous fungi involved in hemicellulose degradation have developed a complex mode of action that includes not only enzymes but also biosurfactants; surface-active molecules that facilitate enzyme-substrate interactions. For this reason, this study aimed to mimic the mechanism of biomass degradation by repurposing plant cell wall degrading enzymes including a cutinase and three esterases to cooperatively contribute to PET degradation. Surfactants of different charge were also introduced in the reactions, as their role is similar to biosurfactants, altering the surface tension of the polymers and thus improving enzymes' accessibility. Notably, *Fusarium oxysporum* cutinase combined with anionic surfactant exhibited a 2.3- and 1.6-fold higher efficacy in hydrolyzing amorphous and semi-crystalline PET, respectively. When cutinase was combined with either of two ferulic acid esterases, it resulted in complete conversion of PET intermediate products to TPA, increasing the overall product release up to 1.9–fold in presence of surfactant. The combination of cutinase with a glucuronoyl esterase demonstrated significant potential in plastic depolymerization, increasing degradation yields in semi-crystalline PET by up to 1.4-fold. The approach of incorporating enzyme cocktails and surfactants emerge as an efficient solution for PET degradation in mild reaction conditions, with potential applications in eco-friendly plastic waste management.



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Scrutinizing biodiversity of lignocellulose-degrading filamentous fungi: extraction and analyses of critical morpho-granulometric parameters

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Keywords: Lignocellulose biomass; filamentous fungi; morphology; morphogranulometry

Lignocellulose biomass is a promising bioresource providing a stable and versatile source of materials for a wide range of application including: renewable energy production, food manufactory, biomaterial production. In order to degrade lignocellulose, filamentous fungi are considered as the most effective biofactory due to their enzyme diversity and high activities, renewable and low-cost advantages. Enzyme production by filamentous fungi performed under submerged condition is associated with important morphological evolution. Controlling fungi complex morphological structure is believed to be one of the main challenges in biotechnology industry. These morphologies directly affect the medium rheology since free mycelia increases culture viscosity and generates non-Newtonian behavior. Compact pellet formation is considered to facilitate biomass separation and secretome extraction in down-stream process; however, the main drawback is mass transfer limitation to the core region leading to cell degradation. The different states of filamentous fungi have a large impact on production yield, maintaining an appropriate morphology for targeted metabolites. In this research, an optical method was developed to characterize the morphology of different filamentous fungi strains by statistical analysis of associated criteria such as: dimension and distribution in submerged culture. Morphological characterizations were performed *ex-situ* using a morphogranulometer (Mastersizer G3S, Malvern Instruments Ltd., software Morphologi v7.21) including standardized microscopic observations, image analysis and statistical treatment of extracted criteria. Three representative filamentous fungi strains (*Talaromyces*, *Aspergillus niger* and *Trichoderma virens)* were selected due to their lignocellulosic biomass degrading capability from the fungal collection provided by FIRI and HUST [1]. Pellet density, shape and size, fiber parameters and associated distribution functions were correlated to biomass in order to describe the diversity of filamentous fungi morphology at different growth stages. Results indicate that under controlled submerged conditions, the three selected strains exhibit different pellets structures – from dispersed mycelia to compact pellet – and with large morphology variations. This first step in understanding filamentous morphology will contribute to correlate morphology with medium rheological properties and to rationalize process up-scale.

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Exploration and expansion of the diversity and characteristics of acidophilic filamentous fungi through environmental sampling and whole-genome sequencing

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Keywords: Acidophile, acidotolerant, extremophile, CAZymes, biodiversity

The study of extremophiles can lead to the discovery of highly tolerant enzymes of value to downstream biotechnology, and deployment of extremophilic filamentous fungi may be an advantage in the degradation of acidic lignocellulosic waste fractions. We recently reported a library of 130 acidophilic and acidotolerant strains of filamentous fungi isolated from Vietnam, including new taxa as well as species not previously associated with acidophilicity [1]. We report here genome assemblies, growth characteristics, and protein classification of six of these recently isolated acidophilic filamentous fungi found in Vietnam, five of which represent new species of Ascomycota (Figure 1). The genome sizes ranged from 27.91-50.08 Mb, and functional annotation predicted 4.4-5.0% CAZyme-encoding genes, suggesting species with highly functional sets of enzymes to degrade biomass. We cultivated three of these species on both xylan and cellulose, at pH 2.5 and 5.5, to determine which enzymes were produced more readily at low pH. The resulting target enzymes may be implicated in the mechanism by which these fungi tolerate extremely low pH conditions, and enable metabolism of polysaccharides.

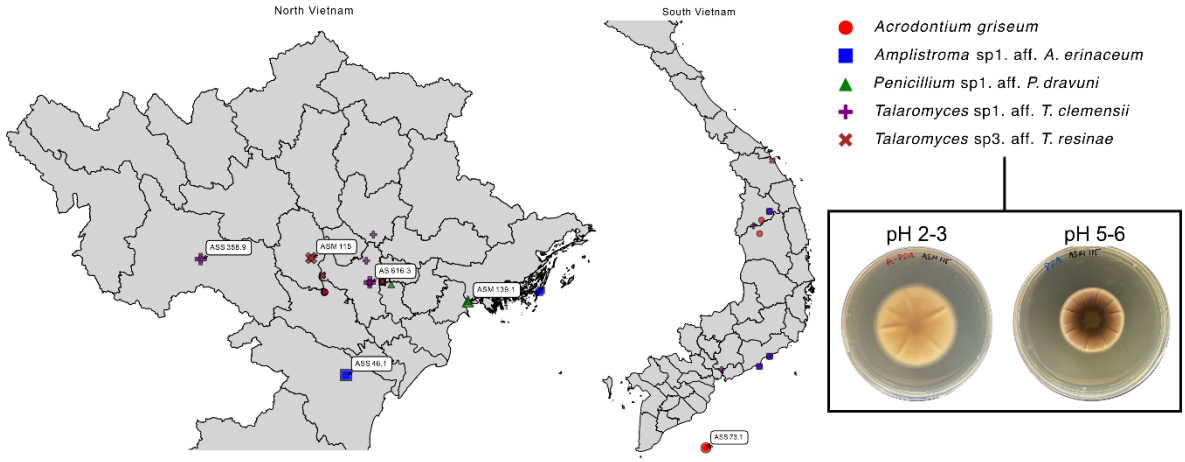

Figure 1. Map showing isolation sites of whole genome-sequenced species, and growth on agar of one species of interest. The labels indicate specific strains taken for WGS.

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Extracting Lignin-Carbohydrate Complexes from Sugar Beet Pulp or Oak to Discover New Enzymes for Cellulose Nanofiber Production

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Keywords: LCC; lignin; cellulose nanofibers; lignin-carbohydrate complex

In the expanding field of biobased materials, cellulose nanofibers (CNF) have received great attention due to its mechanical strength and versatile applications. Sugar beet pulp (SBP), a by-product of sugar production, is a low lignin biomass, making it suitable for CNF production. However, the realisation of a large scale production is hindered by the absence of a fully enzymatic process, as delignification is currently performed through chemical oxidation. Therefore, this work aims to create a lignin-carbohydrate complex (LCC) for enzyme discoveries.

Different types of LCCs will be created, from either SBP or oak. Oak provides a biomass with a much higher lignin content and serves as a natural habitat for white rot fungi, which will later be used for the enzyme discoveries. The extraction will done by ethanol or 훾-valerolactone, and the different LCCs will be compared. The lignin and sugar content and composition will be analysed by Fourier Transform Infrared spectroscopy (FTIR), and Ma-trix-Assisted Laser Desorption/Ionization - Time of Flight (MALDI-TOF). In addition, the LCCs will be analysed by Thin-Layer Chromatography (TLC) and absorbance.

Enzymatic Upgrading of Textile Fibers for a Greener Dyeing Process

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Keywords: surface oxidation, LPMOs, Dyeing, AA9, Lyocell

Lytic polysaccharide monooxygenases (LPMOs) are metalloenzymes that can functionalize several polysaccharides including celluloses and hemicelluloses through their oxidative catalytic cleave of glycoside linkages. LPMOs can be C1-oxidizing enzymes that generate aldonic acids as products, C4-oxidizing enzymes that generate ketoaldoses as products, or C1/C4 oxidizing enzymes that can perform both types of oxidations on various polysaccharides. LPMOs with minimal substrate degradation and high cellulose surface oxidation are critical for functionalizing fibers without degrading much of the fiber itself [1]. Consequently, the increase of aldonic acids on the surface of cellulose, thereby increasing the surface charges can potentially improve its dyeability. In this work, a C1-LPMO, *Dc*LPMO9A from *Delitschia confertaspora*, was used to treat lyocell fibers, semi-synthetic cellulose produced via the lyocell process. LPMO-treated fibers were dyed with Rhodamine 6G for interaction with the LPMO-introduced negatively charged carboxylic acid groups. Notably, color retention, fastness, and density were significantly increased with increased loading of *Dc*LPMO9A treatments, with the highest being observed at 1% (of fiber weight) loading. These results indicate the viability of LPMOs usage in an amorphogenic capacity and underscore the importance of increased research focus on LPMOs as biopolymer upgrading tool.

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Synergy between endoglucanase and fungal expansin-like proteins studied by quartz crystal microbalance with dissipation

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Keywords: Expansin; Endoglucanase; QCM-D; Synergy; CNF

Expansins were originally found from plants, where they loosen the plant cell wall during cell wall formation without hydrolytic activity [1]. Microbial proteins that have similarities to plant expansins are called expansin-related proteins, and they include expansin-like proteins. Plant expansins and expansin-like proteins contain two domains: N-terminal domain I that is related to the catalytic domain of glycoside hydrolase-45 and C-terminal domain II that belongs to carbohydrate binding module family 63 [2]. Plant expansins and expansin-like proteins are hypothesized to disrupt the non-covalent bonding between cellulose and hemicellulose. However, the mode of action is not well understood. Bacterial expansins have been more characterized than fungal expansins, and a lot of the research has focused on the characterization of *Bacillus subtilis* expansin-like protein (*Bs*EXLX1). The goal of this research is to bring more light on the action of fungal expansin-like proteins using quartz crystal microbalance with dissipation (QCM-D).

Two different fungal expansin-like proteins were produced in *Pichia pastoris*: Expansin-like protein from *Aureobasidium pullulans*, *Apu*EXLX1, and expansin-like protein from *Allomyces macrogynus*, *Ama*EXLX1. The adsorption of the proteins was first investigated with pull-down assays where multiple substrates and pH-values were tested. The adsorption of the proteins was further investigated using QCM-D. The two proteins showed different adsorption behavior based on the pull-down assays and QCM-D. QCM-D was also used to study the enzymatic hydrolysis of cellulose nanofiber (CNF), and whether the expansin-like proteins could improve the action of endoglucanase. Both proteins seemed to work synergistically with endoglucanase and improve the hydrolysis of CNF.

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Unravel the role of unspecific peroxygenases in lignin modifications

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Keywords: Unspecific peroxygenases, H_2O_2 concentration, pH, product specificity, Cα-Cβ oxidation

Unspecific peroxygenases (UPOs, EC 1.11.2.1) are heme-thiolate enzymes that utilize H_2O_2 as an electron acceptor and as a direct oxygen source to introduce an oxygen atom into a substrate. These enzymes are particularly intriguing due to their substrate promiscuity; UPOs exhibit catalytic activity across a range of compounds, including fatty acids, naphthalene derivatives, styrene, and various aromatic substances [1]. UPOs are abundant in lignocellulose-degrading fungi, they are expressed and secreted during the lignin degradation phase of fungal colonization, alongside other lignolytic enzymes such as laccase and peroxidases [2].

Despite their prevalence, it is not known if and how they participate in fungal lignin conversion, and the physiological role of UPOs remains elusive. This project aims to unravel if UPOs can catalyze modifications on lignin, to map what such modifications are, and to steer reactions towards a defined set of reactions. We hypothesize that UPOs are capable of inducing oxidative modifications to lignin that can improve utilization.

To investigate this, four distinct UPOs from ascomycetes *Chaetomium globosum* and *Podospora anserina*, were heterogeneously expressed in *Pichia pastoris*.

The peroxidase versus peroxygenase behavior of all four UPOs show different propensity for either of the two reactions, and show a strong pH and H_2O_2 concentration dependency. Additionally, the UPOs' activity on lignin-like compounds such as veratrylglycerol β-guaiacyl ether indicate the ability to catalyze hydroxylations, demethoxylations, Cα oxidation, Cα-Cβ oxidation and traces of β-O-4'-bond breakage. Ongoing investigations delve into the activity of UPOs on polymeric lignin.

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Changes in elemental composition of lignin during enzymatic modification by white rot fungi

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Keywords: white rot fungi, laccase, MnP, elemental analysis, kraft lignin

Fungal peroxidases and laccases are enzymes known for their ability to oxidize hydroxyl groups. These enzymes induce various reactions in lignin such as phenol oxidation, Cα-oxidation, aryl-alkyl cleavage, Cα-Cβ cleavage and condensation of lignin. In this study, we explored the use of a white-rot fungal secretome for biodesulfurization of kraft lignin. Our findings revealed that fungal enzymes effectively cleaved sulfur bonds attached to α- and β-carbons, resulting in approximately a 25% reduction in sulfur content. Additionally, we investigated the impact of copper supplementation on enzyme expression and lignin modification. The changes in lignin structure were attributed to the combined activity of several enzymes, emphasizing the importance of studying enzyme combinations rather than relying solely on individual enzymes for lignin modification. Furthermore, the condensed high molecular weight kraft lignin produced during this process holds promise as a starting material for carbon fibers and electrodes, with the removal of sulfur enhancing its applicability.

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Overexpression of the Transcription Factor HAA1 to enhance *Komagataella phaffii* resistance to inhibitory compounds

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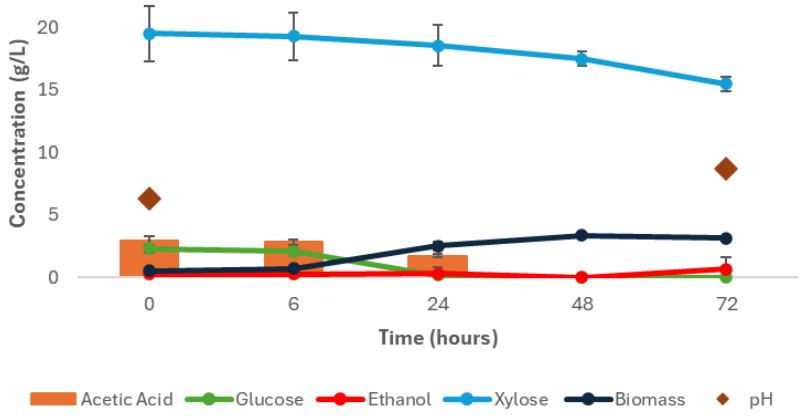
Keywords: *Komagataella phaffii*, Lignocellulosic Biomass, Genetic Engineer, Transcription Factor.

The use of renewable feedstocks, like lignocellulosic biomass hydrolysates, is growing rapidly in industrial biotechnology. Unfortunately, one of the biggest obstacles for this industry is the microorganism response to inhibitory compounds found in renewable biomasses, such as acetic acid and lignin derived aromatic compounds. Those can inhibit or even completely block the cell's metabolic activity.

In the past, genetically engineered strains of *Saccharomyces cerevisiae* have shown high tolerance to lignocellulose-derived inhibitors. This work aimed to evaluate the inhibitor tolerance of *Komagataella phaffii* engineered for HAA1 overexpression. This yeast has been employed in several bioprocesses and gained attention for the production of bio-based chemicals (Moraes et al. 2024).

The overexpression of the transcription factor *HAA1* improves the yeast's resistance to acetic acid and lignocellulose hydrolysate. Interestingly, yeast cultivation allowed increased consumption of acetic acid and a rise in extracellular pH, as shown in Figure 1.

Figure 1: Fermentative profile of the lineage X33_pGAP_HAA1 in sugar cane bagasse hydrolysate medium.



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SILANIZED HEMP BIOMASS AFTER HYDROLISIS PROCESS AS A COMPOSITE FILLER

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Keywords: hemp, biomass, silanization, biocomposites, flammability

Nowadays composites are used increasingly in place of raw polymers. The growing popularity composites owe to a lower price or improved properties. Among the composites with various kinds of fillers, more and more popular are fillers of natural origin. Filling polymers with renewable lignocellulosic material affects not only the reduction of production costs, but it makes it more environmentally friendly at the same time which is the main reason for their wider use in various industries.

The research is aimed at understanding the interaction between modified biomass with polymer matrix. To achieve the goal set in the studies chemical modification of natural fibres will be carried out. It aims to increase compatibility with the biocomposites polymer matrix and to reduce the flammability of lignocellulosic material. Used for this purpose the organo silicon compounds have ability to produce additional bonds which should reinforce the composite structure. Furthermore, because the chemistry of silicon is similar to carbon chemistry, aside from functional groups that binds silicon compounds to the fibre surface and silanes among them, there is a possibility of adding the fourth group, which can interact with used halogen-free flame retardants.

Modified natural fibre will be prepared in water-alcohol solutions of silanes. After silanization process fibres will be dried, cross linked and introduced into the biopolymer matrix using the laboratory extruder Dynisco LME. Flammability test will be carried out using the method of pyrolysis and combustion microcalorimeter (PCFC).

Examined will be the impact of conducted biomass modifications on its thermal stability and flammability as well as impact of modified biomass on the composites properties. The result of research will be knowledge of a chemical methods of biomass modifications, as well as fire retardant impact of cellulose material on the obtained biocomposites.

The study has been carried out within the Project – How the chemical composition of the waste biomass from hemp (Cannabis sativa L.) affects its usability in bioproducts?, financed by National Science Centre Poland, SONATA 17 project no. 2021/43/D/NZ9/01215.

COMPARISON OF THE LIGNOCELLULOSE STRUCTURE OF BIOMASS IN MONOECIOUS AND DIOECIOUS FORMS OF HEMP

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Keywords: lignocellulose structure, hemp biomass, monoecious and dioecious hemp, bioethanol

Fiber hemp is an annual cultivated species that is a source of a large amount of raw material for the bioproducts. This is caused by the very high yield potential, which for straw yield is over 15 t/ha. An interesting and rational proposition for its use may be, obtaining advanced biofuels.

The presented research consisted in determining the influence of the variety and botanical form on the quality of the obtained hemp biomass, as a source for bioethanol production. For this purpose, the pretreatment process and enzymatic hydrolysis, as steps necessary to obtain bioethanol were conducted. Then the structure and morphology of lignocellulosic biomass was examined. The plant material consisted of 2 monoecious and 2 dioecious hemp varieties, characterized by high total yield. The tested genotypes were selected based on the results of a comparative field experiment including 20 varieties of different geographical origin. Significant differences were found between the tested varieties in terms of yield structure traits. In the second part of the research, alkaline pretreatment of selected varieties of hemp biomass using NaOH was carried out to loosen the compact lignocellulose complex and enzymatic hydrolysis was carried out to increase the specific surface area of cellulose so that its chains were available to cellulolytic enzymes. The optimal parameters for enzymatic hydrolysis were determined by RSM (response surface methodology) method.

It was concluded that the variety has a strong, individual impact on the chemical structure of hemp biomass. The influence of individual stages of hemp biomass conversion on its structure and morphology was also demonstrated.

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Phenolic compounds and phenol-oxidases activities as markers for bacterial behaviour during lignin utilization

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Keywords: Valorization, peroxidases, laccases, metabolomics, UHPLC-ESI-HRMS

Lignin is a renewable source of aromatic molecules which is present in large quantities throughout the world in plant biomasses and as by-products of certain industries (paper industry, wood industry,...). Lignin is mainly burned and used to produce energy because of its complex structures and chemical compositions. Lignin can be degraded and transformed by microbial and enzymatic processes known to be respectful of the environment. Biological valorization remains challenging as biocatalysts are not sufficiently effective and efficient. Moreover, the complexity and heterogeneity of lignins are a barrier to their use. Understanding the microbial behavior on lignins by finding some markers of their efficient transformation could lead to the development of effective biological routes to valorize the aromatic moieties of these polymers.

In Nature, several micro-organisms are able to degrade lignins [1]. Ligninolytic bacteria have some interesting features in term of ligninolytic enzymes productions, utilization of aromatic compounds as carbon source, their metabolization *via* various intracellular pathways and finally some productions of molecules of interest [2]. In this work, we use multiple approaches (growth kinetics studies, ligninolytic activities quantification, IRTF and metabolomic studies by ultra-high performance liquid chromatography - electrospray ionisation - high-resolution multiple-stage tandem mass spectrometry (UHPLC-ESI-HRMS)) to study the behaviour of two ligninolytic bacteria *Pandoraea norimbergensis* [3] and *Comamonas composti* [4], during their growth on lignins with different structures and origins (Kraft and Protobind lignins). Results showed that growths profiles, lignin modifications, consumption and production of phenolic monomers and oligomers vary within bacteria and according to the lignin used. The targeted bacteria behave differently on the two lignins. This study demonstrates that the efficient transformation of lignins requires a suitable combination of biocatalysts (bacteria)/lignins. Micro-organisms used must be selected on the basis of their metabolic capacities, as well as the structure and composition of the substrates to be valorized.

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Changes in the enzyme profile of the white-rot fungus *Bjerkandera adusta* in co-culture with the brown-rot fungus *Gloeophyllum trabeum.*

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Keywords: endocellulase; xylanase; peroxidase; wood-rotting fungi

The coculture strategy, which can simulate the relationship of microorganisms in nature and activate silenced gene clusters through the interaction between strains, has been widely used to unlock unknown biosynthetic potential. The present study investigated the interaction between the white-rot fungus *Bjerkandera adusta* and the brown-rot fungus *Gloeophyllum trabeum* in the growth and production of lignocellulolytic enzymes by co-culture in solid and liquid medium. In order to study the compatibility between the species, the growth in solid medium was first evaluated, where it was possible to observe a mixed mycelial growth of both fungi, without pigment production at the interface of the mycelia, indicating a partial compatibility. In addition, changes in lignocellulolytic enzymes in solid and liquid media were observed in cocultures of *B. adusta* and *G. trabeum* compared to monocultures. The characterization of the enzyme fungal cocktails produced in coculture provided detailed insight into the interactions and influence of *G. trabeum* on enzyme profile of *B. adusta*. A significant increase in the activity of oxidative enzymes was observed in the coculture, particularly peroxidase, which showed a 2-3-fold increase in the coculture compared to *B. adusta* monoculture. These results suggest a stimulatory effect of *G. trabeum* (which does not produce peroxidases) on *B. adusta* in coculture. Conversely, in the case of cellulolytic and hemicellulolytic enzymes, significant inhibition was observed due to the effect of coculture compared to *G. trabeum* monoculture. Electrophoresis (zymogram) confirmed the presence of peroxidases only in *B. adusta* and coculture and their absence in *G. trabeum*, as well as the presence of xylanases only in *G. trabeum*. Purification by ion exchange chromatography allowed the separation of different protein fractions, some of which showed endoglucanase activity (in all fungal cultures) and peroxidases (in *B. adusta* monoculture and coculture). These results contribute to the understanding of fungal interactions in the production of lignocellulolytic enzymes and highligh the potential of coculture to increase the production of oxidative enzymes in *B. adusta* for various biotechnological purposes, currently being applied in dye decolorization.

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Structural Characterization of Plant Polysaccharide Synthase Complexes

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Keywords: Cellulose Synthase Complex, Cryo-EM Tomography, Enzyme Engineering, Tailor-made plant biomass

Cellulose and other polysaccharides of the plant cell wall are major renewable resources in the world with enormous biotechnological potential. Cellulose synthesis, driven by the cellulose synthase (CESA) complex (CSC), is pivotal for plant cell wall formation and represents a key renewable resource [1]. Our research integrates cryo-electron microscopy (cryo-EM) to explore CSC architecture *in planta*, focusing on engineered cellulose synthase variants designed for enhanced biomass properties. Additionally, we investigate microtubule-associated proteins (MAPs) linked to the CSC, known for their role in stress resilience (e.g., high salt stress), to elucidate their impact on cellulose synthesis regulation and plant morphology [2]. This interdisciplinary approach combines structural biology techniques with computational modeling to advance biotechnological applications in biomass valorization. Recent findings also highlight the role of CSC in response to phosphate starvation, showing increased activity at the plasma membrane under low-phosphate conditions, which modifies root growth by altering cell wall structure [3]. Furthermore, we discuss the essential role of the NKS1/ELMO4 protein complex in maintaining middle lamellae integrity and cell adhesion, crucial for agricultural characteristics such as fruit ripening and organ abscission [4].

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Discovery of new lignocellulose-degrading enzymes by a metatranscriptomic approach

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Keywords: Metatranscriptomics, CAZyme, lignocellulose, cellulases, LPMO

Enzymatic hydrolysis of some lignocellulosic substrates such as Miscanthus, pine or poplar wood is still too inefficient for industrial production of second generation biofuels and biobased chemicals. In order to valorize these biomass materials, enzymatic cocktails able to efficiently decompose these recalcitrant substrates are therefore needed. In the present work, metatranscriptomic libraries were constructed from plant biomass degrading environments such as forest litter and horse dung. This approach allows targeting eucaryotic genes which could potentially be used for complementation of enzymatic cocktails produced by filamentous fungi such as *Trichoderma reesei*. In total, three libraries were sequenced, assembled using three different algorithms and submitted to CAZyme (Carbohydrate-Active enZymes) annotation. The CAZy classification system which groups sequences into different enzyme classes and families according to sequence and structural similarities allows prediction of their activities and identifying enzymes of interest. Between 111.000 and 165.000 partial or full-length ORFs could be retrieved depending on the library and the assembler used, and 0,3 – 1,7 % of these sequences were identified as glycoside hydrolases (GH). Interestingly, forest litter libraries were much more divers than the one originating from horse dung which constitutes an ecological niche. GH families containing beta-1,4-glucanases and AA (auxiliary activities) families representing cellulose-active LPMOs were analyzed in more detail. The big majority of these sequences were affiliated to a wide variety of genera belonging to the Ascomycota and Basidiomycota phyla and some of them displayed less than 50 % identity with known sequences from databases. In addition, the results showed that full-length sequences could be recovered, thus demonstrating the potential of this method for new industrial enzyme discovery.

Polyphenol Oxidase Activity on Lignin-Units

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Keywords: polyphenol oxidase; lignin; hydroxylation; demethoxylation; methoxy-*ortho*-diphenol

The natural heterogeneity of guaiacyl (G) and syringyl (S) compounds from lignin processing hampers their direct use as plant-based chemicals and materials. Here, we investigate a new group of fungal short polyphenol oxidases (PPOs) with the potential to reduce the heterogeneity of lignin-derived mixtures. We characterized six short PPOs from lignocellulose-degrading ascomycetes (*Myceliophthora thermophila*, *Chaetomium globosum*, and *Parascedosporium putredinis*) for their ability to react with G- and S-type model phenolic compounds. All six short PPOs catalyze the *ortho*-hydroxylation of G-compounds (guaiacol, vanillic acid, and ferulic acid), forming methoxy-*ortho*-diphenols, while a subset also acts on S-compounds (syringol, syringic acid, and sinapic acid), generating products identical to those obtained with G-type substrates. Assays with $^{18}O_2$ show that these PPOs catalyze *ortho*-hydroxylation and *ortho*-demethoxylation of S-compounds, generating methanol as a coproduct. This indicates a distinct catalytic mechanism compared to that of bacterial aryl O-demethylases. Adding a reductant can steer the PPO reaction to form methoxy-*ortho*-diphenols instead of unfavorable quinones that lead to polymerization. We have also investigated the PPOs´ copper loading process. Heterologous expression in *P. pastoris* produces enzymes with copper-deficient active sites, often replaced by other divalent cations, as suggested by ICP measurements. To address this, we developed a copper-saturation method for the purified enzymes and achieved near-complete copper loading of PPOs. The presence of a type-3 copper center typical of PPOs was confirmed with UV-vis spectroscopy, with the appearance of a distinct band at 345 nm upon H_2O_2 addition, a peak characteristic of the oxy-state of PPOs. Moreover, copper loading increases the thermal denaturing midpoint of one of the PPOs, suggesting that the occupancy of the active site by copper ions may contribute to the enzyme's thermal stability. *Mt*PPO7 from *M. thermophila* and *Cg*PPO-473 from *C. globosum* display denaturing midpoints of c.a. 75°C and 60°C, respectively, indicating stable enzymes at biorefining process conditions. The application of fungal short PPOs opens new routes to reduce the heterogeneity and methoxylation degree of mixtures of G and S lignin-derived compounds, presenting unique opportunities for lignin valorization.

Recombinant AA14 from *Trichoderma harzianum* and its collaborative effect with GH30 from *Thermothielavioides terrestris* for xylooligosaccharides production using sugar cane bagasse as raw material.

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Keywords: LPMO; xylooligosaccharides; biomass valorization; hemicellulases; fungal platform

LPMOs are enigmatic enzymes that have revolutionized the way we understand the destructuring of plant biomass. Among its 17 families, family 14 (AA14) is perhaps one of the most controversial and least known due to the difficulty in detecting its activity in the structural carbohydrates that make up plant biomass. In this work, a new AA14 from *Trichoderma harzianum* (ThAA14) has been produced heterologously, and its collaborative effect is described acting with a GH30 from *Thermothielavioides terrestris* (TtXyn30) for the production of xylooligosaccharides from sugar cane bagasse (SCB). Both enzymes were produced in *Aspergillus nidulans* and characterized biochemically, including the production of H_2O_2 by ThAA14. Although it was not possible to detect the activity of ThAA14 on various polymeric substrates rich in hemicellulose using HPAEC-PAD, the collaborative effect of ThAA14 and TtXyn30 acting on SCB subjected to ASE (alkaline sulfite ethanol) pretreatment was evaluated. The simultaneous action of enzymes allowed increases of more than 10 percent in the production of xylose and xylooligosaccharides compared to the same products obtained using the enzymes separately. This work, in addition to describing new enzymes, allowed us to verify their application to obtain value-added products as a strategy for valorizing residual plant biomass.

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Enzymatic Hydrolysis at High Solids Loading of Deacetylated Rice Straw

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Keywords: Rice straw; deacetylation; saccharification; enzyme loading; high solids.

Abstract

Rice straw is considered a promising feedstock for 2G ethanol production in biorefineries [1]. However, one of the main challenges is to produce high concentrations of fermentable sugars from biomass [2]. In this study, we evaluated the saccharification efficiency at high solids (20% w/v) of rice straw deacetylated with dilute alkali. Assays were carried out for 49 hours at 50°C using Cellic Ctec2 SAE0020 at different enzyme loads (ranging from 10 to 45 mg protein/g cellulose) at pH 4.8 and 100 rpm. It was shown that, in Erlenmeyer flasks, increasing enzyme dosage led to a ~46% increase in glucan and xylan conversion, achieving maximum conversions of 72% and 52%, respectively, at a load of 30 mg protein/g cellulose. Under these conditions, the total fermentable sugars reached 98 g/L. As a strategy to improve hydrolysis at high solids, a novel reactor design (vertical ball mill reactor) was assessed [3]. At 30 mg protein/g cellulose, glucan and xylan conversion in the reactor improved to 85% and 62%, respectively, producing 117 g/L fermentable sugars. This study showed that reactor design plays a key role in the enzymatic hydrolysis step at high solids loading. Based on the results, further studies will be undertaken to develop an adequate enzyme formulation, especially with complementary activities (e.g., xylanases), aiming to improve hydrolysis yield.

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Sequence-functional investigations of expansin-related proteins that modify cellulosic materials

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Expansins are non-lytic proteins found in plants and microbes which contribute to the weakening of lignocellulosic networks. These proteins consist of two domains, an N-terminal domain I that adopts a double-ψ-β-barrel (DPBB) fold and is related to the catalytic domain of glycoside hydrolase-45 (GH45), and a C-terminal domain II that belongs to carbohydrate binding module (CBM) family 63. Expansinrelated proteins of microbial origin include fungal loosenins that comprise the domain I structure and lack the CBM63 containing domain II. The current study investigates sequence-functional relationships of recombinantly produced and characterized expansin-related proteins from diverse fungal origin. Protein features such as pI, surface charge distribution and hydrophobicity were calculated from the sequence. The aim was to determine if these features drive specific protein binding to chitin, cellulose, xylan and hardwood pulp. Recent discoveries connecting predicted protein pI and surface charge to binding activity will be presented.

Enzyme-mediated transformation of lignocellulose-derived 5-hydroxymethylfurfural to the precursor for polymer synthesis 2,5-furandicarboxylic acid

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Keywords: biocatalysis, oxidase, furans, HMF, FDCA

Furans, including 5-hydroxymethylfurfural (HMF) and its oxidized derivative 2,5-furandicarboxylic acid (FDCA), are promising compounds that can be used as precursors for polymer synthesis due to their high chemical reactivity. Producing these compounds from sugar streams derived from lignocellulose presents a promising alternative to fossil fuels, allowing for the conversion of biomass into higher-value chemicals [1]. This study focuses on exploiting a mild and regioselective biocatalytic process using novel fungal enzymes for the biotransformation of HMF and its oxidative derivatives into valuable monomers like FDCA. Two specific enzymes, one with glyoxal oxidase activity (*Gl*GlyOx) and another with galactose oxidase activity (*Fo*GalOx), were identified from the Auxiliary Activity AA5 family (AA5) of the CAZy database, while an aryl alcohol oxidase (*Gl*AAO) from the AA3 family was also selected for further study. These genes were expressed heterologously in the methylotrophic yeast *Pichia pastoris*, and the enzymes were purified and characterized biochemically. The enzymes were tested for their ability to transform furans into oxidized derivatives, both individually and in combination with horseradish peroxidase (HRP), as well as *in-house* produced fungal unspecific peroxygenases (UPOs). *Fo*GalOx was found to catalyze the conversion of various furanic compounds, while *Gl*GlyOx specifically oxidized HMF to 5-hydroxymethyl-2-furancarboxylic acid (HMFCA) and furan-2,5-dicarbaldehyde (DFF) to 5-formylfurancarboxylic acid (FFCA). *Gl*AA efficiently oxidized HMF to FDCA, while the presence of HRP and UPOs significantly enhanced these reactions. The addition of catalase, which consumed excess hydrogen peroxide, further improved the process by preventing adverse effects from its accumulation. Overall, the results highlight the potential of these enzymes to oxidize furan substrates from lignocellulosic biomass, producing high-value compounds with diverse industrial applications.

[1] Dedes A. et al., "Novel routes in transformation of lignocellulosic biomass to furan platform chemicals: from pretreatment to enzyme catalysis", Catalysts, vol. 10, no. 7, pp. 743, 2020.

Investigation of bacterial expansin loosening of chitinous materials

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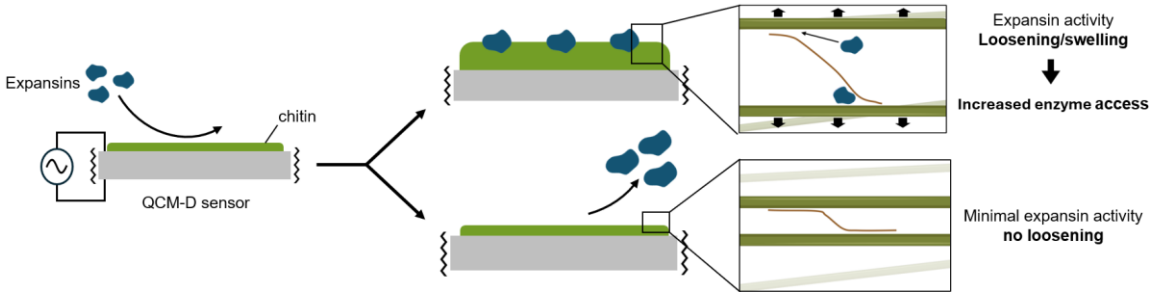
Keywords: expansin, chitin, QCM-D, non-lytic, protein



After lignocellulose, chitinous materials represent the second most abundant renewable bioresource in the world; nevertheless, chitin remains underutilized. Its recalcitrant nature and limited solubility present a challenge to establishing cost efficient routes to chitin valorization. Expansins are non-lytic proteins that have been shown to non-covalently loosen lignocellulosic structures by disrupting hydrogen bonding, although their exact molecular mechanism is unclear [1]. Substrate loosening by expansins can increase enzyme accessibility to the material, thereby enhancing enzyme activity and potentially reducing enzyme load and cost [1]. Expansin applications for cellulose deconstruction have been well documented, making the structurally comparable biopolymer chitin a rational next candidate.

This research investigates the action of microbial expansins on chitinous substrates using quartz crystal microbalance with dissipation monitoring (QCM-D). QCM-D is a powerful research tool capable of measuring real-time nanogram-scale changes of a sensor coated with a substrate (such as chitin) upon expansin treatment. Combined, these measurements can correlate to changes in quantity and mechanical properties of a chitin coating, providing insight into expansin loosening activity at the molecular level.

Three expansin candidates with diverse structural modularity have been expressed in *Escherichia coli* and purified. The trimethylsilylation of chitin from crab shells was performed to permit its coating on silicon oxide QCM-D sensors; after coating, the chitin was regenerated to its native form using hydrochloric acid vapour. The successful regeneration of the chitin thin film was monitored using XPS. The chitin coated sensors were subjected to expansin treatment, and changes in dissipation and frequency were measured and correlated to the degree of swelling of the chitin substrate. This work presents a comparative analysis of microbial expansin action on chitin to expand our understanding of substrate preference within this ubiquitous protein family.

[1] D. J. Cosgrove, "Microbial Expansins," *Annu. Rev. Microbiol.*, vol. 71, pp. 479-497, 2017.

EFFECT OF LIGHT WAVELENGTHS ON THE PRODUCTION OF TOTAL CAROTENOIDS BY Rhodotorula mucilaginosa USING A RICE HUSK HYDROLYSATE AS CARBON SOURCE

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Keywords: biopigment, yeast, visible wavelengths, carotenoids, lignocellulosic biomass

A sustainable alternative for obtaining biopigments is through the metabolism of different microorganisms, making the production process of biopigments more feasible and competitive with synthetic compounds. Carotenoids, pigments considered photoprotective, are produced in yeast in response to oxidative stress, such as that caused by light exposure. This research aimed to enhance the production of total carotenoids by Rhodotorula mucilaginosa through direct exposure to lights with different visible wavelengths during batch fermentation. The yeast was grown in a synthetic and rice husk hydrolysate medium containing 30 g/L glucose for 72h. Then, 0.1 g of dried cells were used for biopigment recovery by an ultrasonic-assisted extraction. The extracts were analyzed by spectrophotometry, and the color of the cells (before extraction) was analyzed by a colorimeter (CieLab analysis). According to the results, the incidence of white, green, red, yellow, and blue lights during the cultivation of R. mucilaginosa for 72 h did not inhibit the growth of the yeast. Except for blue light, all other light exposures increased biopigment production by at least two-fold. The highest pigment production was achieved under red and yellow light exposure. In addition to the increased total carotenoid production, differences were observed in the wavelengths detected by cell color analysis. Higher peaks were detected at wavelengths between 360-450 and 450-580 nm when the yeast was grown under dark blue light conditions and other visible wavelengths, respectively. These findings suggest that some visible light wavelengths can regulate carotenoid production, with dark and blue conditions potentially inducing the production of other pigmented molecules. Furthermore, the CieLab analysis revealed that the a*(greenish or reddish) and b*(blueish or yellowish) values varied with light exposure, except for blue light. The cells appeared more reddish under white, green, red, and yellow lights than in the dark and blue light conditions. These results aligned with the observed increase in total carotenoid production. In conclusion, the production of biopigments from R. mucilaginosa is strongly influenced by light exposure during fermentation, with the highest increase in carotenoid production under yellow and red-light wavelengths. This research introduces a promising strategy to enhance carotenoid production and opens a wide range of alternatives for the application of this physical parameter to different bioprocesses.

Harnessing the catalytic potential of a ferulic acid esterase for MHET hydrolysis

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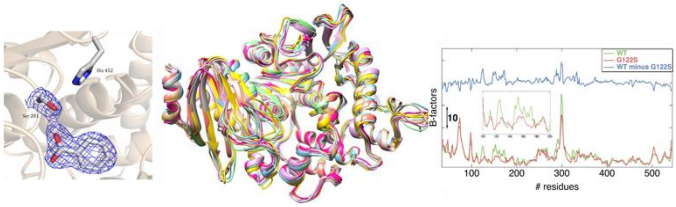
Keywords: plastic degradation, MHETase, feruloyl esterase, X-ray crystallography, docking

The enzymatic breakdown of plastics presents a promising approach to address their uncontrolled accumulation on Earth. Polyethylene terephthalate (PET) is a widely used polymer used in packaging, construction, and agriculture. Since 2000, numerous enzymes, capable of decomposing plastic, such as lipases and carboxyl ester hydrolases have been discovered: PETases target the polymer's ester bonds, producing mono-(2-hydroxyethyl) terephthalate (MHET) as the primary water-soluble degradation product and MHETases then cleave the ester bonds of MHET, yielding terephthalic acid (TPA) and ethylene glycol (EG). Ferulic acid esterases, and specifically those belonging to tannase-like family, are structural homologs of the well-studied bacterial MHETase from *Ideonella sakaiensis*, while their primary role in nature is to cleave the ester bonds between hydroxycinnamic acids and arabinose in the plant cell wall. We have previously demonstrated that *Fo*FaeC, a tannase-like feruloyl esterase, shows activity on PET oligomers as well as synergistic effect for PET degradation, when combined with PETases[1]. In the frame of the present work, an *Fo*FaeC variant, G122S, was created by structure-guided mutagenesis, in an effort to mimick MHETase active site. Compared to wild-type *Fo*FaeC, G122S variant exhibits increased catalytic activity against MHET. The crystallographic structure of both wild-type *Fo*FaeC and G122S variant were used for docking simulations aiming to acquire deeper understanding and interpretation of these biochemical findings. Shedding light on the structural determinants of PET-active enzymes will allow the production of robust biocatalysts for plastic degradation.



This research was implemented in the framework of H.F.R.I call "Basic research Financing (Horizontal support of all Sciences)" under the National Recovery and Resilience Plan "Greece 2.0" funded by the European Union – NextGenerationEU (H.F.R.I. Project Number: 15024).

[1] G. Taxeidis, E. N ikolaivits, J. N ikodinovic-Runic and E. Topakas, "Mimicking the enzymatic p lant cell wall hy-drolysis mechanism for the degradation of p olyethylene terephthalate" Environmental Pollution, vol. 356, 124347, 204

Unravelling the structure-function of a tandem pair of glycoside transporters in the marine flavobacterium *Zobellia galactanivorans* Dsij^{T}

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Keywords: glycan transporter, SusC/D, marine Bacteroidota, algal/plant biomass

Members of the Bacteroidota phylum are considered primary degraders of polysaccharides. A unique feature of Bacteroidota genomes is the presence of **Polysaccharide Utilization Loci** (PUL) which are clusters of co-regulated genes encoding a complement of cell Surface Glycan-Binding Proteins (SGBPs, including the **SusD-like**), TonB-dependent transporters (**SusC-like**), Carbohydrate Active Enzymes (CAZymes) and carbohydrate sensors/ transcriptional regulators. Among the 73,782 PULs identified by the PULDB tool from the CAZy database, only three SusCD transporters have been structurally characterized so far [1, 2, 3]. These limited data revealed that the SusCDs mediate substrate uptake via a pedal bin mechanism. Nevertheless, many features of SusCD function in glycan uptake remain unclear. The marine flavobacterium ***Zobellia galactanivorans*** **Dsij^{T}** is a model organism for the bioconversion of algal polysaccharides [4], for which genomic and transcriptomic data are available, as well as genetic tools. We have identified a PUL that contains an adjacent pair of SusCD genes, defined as **tandem repeat SusCD** (trSusCD). While trSusCD are present in only around 3% of the encoded PULs in the phylum Bacteroidota, a recent study suggests that they provide a selective advantage in environments where diverse dominant species compete for similar metabolic niches [5]. Nevertheless, the function and structure of trSusCD proteins have never been investigated so far. Besides, preliminary data obtained on *Z. galactanivorans* suggest that the trSusCD could target different substrates, as the PUL is upregulated in presence of laminarin, mixed-linked glucan and xylan [6]. This substrate versatility would expand our perceptions regarding PUL machineries, which so far have demonstrated gene organization that suggests one cognate PUL for each substrate type. Investigating the structure-function relationships of these proteins by combining different approaches (genetics, biochemistry, cryoEM) will provide novelty and a better understanding of PULs functionality.

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[5] Gálvez *et al*. (2020) Cell Host & Microbe
[6] Thomas *et al*. (2017) Frontiers in Microbiology.

Discovery of alkaline laccases from basidiomycete fungi through machine learning based approach

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Keywords: machine learning, alkaline laccase, pH optimum, prediction, basidiomycete fungi

Laccases can oxidize a wide range of substrates and offer promising applications in various industrial sectors. However, the discovery and optimization of laccases with desirable pH optimum remains a challenge due to the labor-intensive and time-consuming nature of traditional laboratory methods. This study presents an integrated machine learning (ML) approach for predicting pH optima of basidiomycete fungal laccases, using a small, curated dataset against a large metagenomic dataset. Comparative computational analyses revealed structural and pH-dependent solubility differences between acidic and neutral-alkaline laccases, helping us to understand the molecular basis of the enzyme pH optimum. The pH profiling of the two selected ML-predicted candidate alkaline laccases from the basidiomycete fungus *Lepista nuda* further validated our computational approach and demonstrated the accuracy of this comprehensive method. This study demonstrates the efficacy of ML in predicting enzyme properties from minimal datasets and marks a significant step towards the use of computational tools for the systematic screening of enzymes for industrial and environmental biotechnology applications.

ENZYMATIC HYDROLYSIS OF PAPER PULP PENTOSE SYRUP

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Keywords: Hemicellulases, xylose, pentose syrup, xylanase, xylosidase

The conversion of wood into paper pulp plays a pivotal role in the recovery of wood by-products and is a crucial aspect of the wood science field. However, this activity has been confronted with the digital transition, which has occurred in conjunction with growing economic pressure from emerging countries. As a result, the French pulp and paper sector must now seek new routes to remain competitive. One such route is to diversify wood-derived products based on the concept of biorefineries, in order to fulfil the development of the bioeconomy, which involves more sustainable transformations.

In this context it is important to maximize the monomeric xylose content of pentose syrup stream obtained after wood pre-treatment, the first step of paper pulp production. Indeed, the xylose will serve as a building block for further transformation.

In this study, we evaluated a number of enzymatic cocktails, purified xylanases and xylosidases on a pentose syrup. The most favorable outcomes were observed with a commercial cocktail. To further increase the concentration of xylose, the remaining xylooligosaccharide fraction resulting from the action of the enzymatic cocktail was targeted. The purified hemicellulases were selected and evaluated on the digested pentose syrup, but their enzyme activity was low and there was only a slight increase in xylose concentration. Meanwhile, an immobilization trial was carried out to allow xylose enrichment in a continuous mode and enzyme recycling. In addition, purification steps, including membrane filtration, were implemented in order to increase xylose purity.

The overall performance of hemicellulases in digesting the pentose syrup was found to be relatively unchanged, which suggests that a more detailed characterization of the syrup may prove to be beneficial in guiding the selection of suitable enzymes in future studies.

Did you know ? Publication by ISO of circular economy standards

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Keywords: circular economy, ISO standard, business model

The global economy is "linear" as it is mainly based on extraction, production, use and disposal. This linear economy leads to resource depletion, biodiversity loss, waste and harmful losses and releases, all of which collectively are causing serious damage to the capacity of the planet to continue to provide for the needs of future generations.[1] Moreover, several planetary boundaries have already been reached or exceeded.

There is an increased understanding that a transition towards an economy that is more circular, based on a circular use of resources, can contribute to meeting current and future human needs (welfare, housing, nutrition, healthcare, mobility, etc.). Transitioning towards a circular economy can also contribute to the creation and sharing of more value within society and interested parties, while natural resources are managed to be replenished and renewed and in a sustainable way, securing the quality and resilience of ecosystems.

Organizations recognize many potential reasons to engage in a circular economy (e.g. delivering more ambitious and sustainable solutions; improved relationships with interested parties; more effective and efficient ways to fulfill voluntary commitments or legal requirements; engaging in climate change mitigation or adaptation; managing resource scarcity risks, increasing resilience in the environmental, social and economic systems), while contributing to satisfying human needs.

The ISO 59000 family of standards (see Figure 1) is designed to harmonize the understanding of the circular economy and to support its implementation and measurement. It also considers organizations, such as government, industry and non-profit, in contributing to the achievement of the United Nations (UN) Agenda 2030 for Sustainable Development [2].

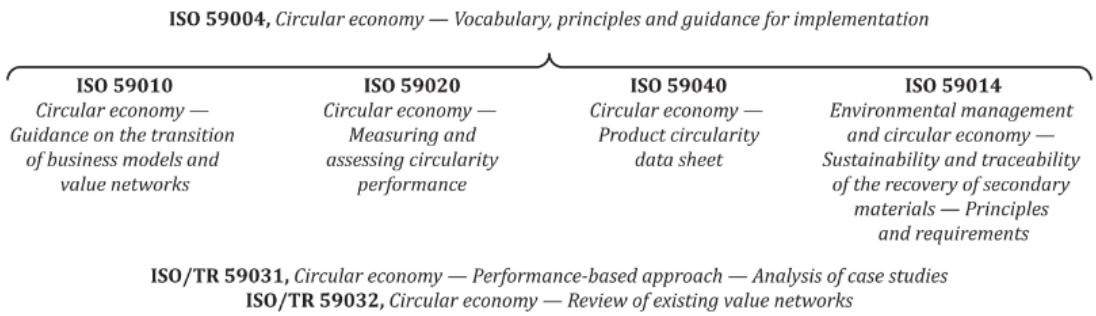

Figure 1 — ISO 59000 family of standards

[1] United Nations. Report of the World Commission on Environment and Development: Our Common Future. The Brundtland Report. United Nations, 1987.

[2] United Nations. Transforming Our World: The 2030 Agenda for Sustainable Development United Nations, 2015.

Bacterial synthesis of bioplastics using lignocellulosic biomass as a carbon source

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Keywords: Polyhydroxybutyrate - Bioconversion - *Paraburkholderia* - Lignocellulosic biomass - Bioplastics - Optimisation

Polyhydroxyalkanoates (PHAs) are bioplastics produced by micro-organisms to store carbon and energy under conditions of nutrient stress[1]. Nowadays, they are of great interest because they could be an alternative to petroleum-based plastics, a non-renewable source of carbon that is at the root of environmental problems. PHAs are currently produced on an industrial scale, but production costs remain high[2]. The high cost of producing PHAs is mainly due to the carbon source used by the micro-organisms for growth, such as simple sugars and hydrolysates derived from the pre-treatment of lignocellulose. Microbial fractionation of lignocellulose is an environmentally sustainable process involving enzymes for plant material degradation[3]. The aim of this study was to optimize PHB production by the *Paraburkholderia madseniana* SEWS6 strain from forest soil using lignocellulosic biomass as a carbon source, in order to reduce the production costs of these biomolecules. The best results were obtained using a sequential batch process on soybean meal, which includes a growth phase followed by a stress phase (nitrogen and phosphorus deficiency). Using this method and compared with a reference strain[4], a PHB production of 1.07 g/L, representing 40% of the dry bacterial pellet, was obtained with the *P. madseniana* SEWS6 strain. The results obtained represent an opportunity to bioconvert lignocellulosic biomass into PHB in a one-step process.

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