CONTENTS

WELCOME TO LIGNOBIOTECH 2018 SYMPOSIUM AND HELSINKI

On behalf of the LignoBiotech Scientific Committee and local organizing team, it is our great pleasure and honor to welcome you to the Lignobiotech 2018, the 5th Symposium of Biotechnology Applied to Lignocelluloses. The symposium focuses on fundamental aspects as well as applications of plant biomass. Due to the active contribution of the participants we have been able to design an interesting and comprehensive programme with seven sessions covering lignocellulolytic enzymes, microbial transformations, analytics and structural aspects of lignocellulose and its constituents, biosynthesis of lignocellulose and material activation, chemicals and fuels from biomass and bioconversion. Besides 8 keynote talks, 30 oral presentations and 60 posters will be presented. On Friday, August 31 we have a special Session, in which three Finnish industries will present examples of lignocellulose utilization. Renewable biomass, the topic of the Symposium, is timelier than ever. We need sustainable solutions for the use of renewable biomass to tackle the grand challenges of climate change and resource sufficiency. The roots of this symposium go back to Reims, France and 2010, when the first LignoBiotech was organized. The meeting was followed by Fukuoka (Japan 2012), Concepcion (Chile 2014) and two years ago by Madrid (Spain). Since the first symposium in 2010 we have witnessed substantial development. Thanks to active research and development, the bioeconomy is becoming an integral part of our everyday lives in the areas of energy, chemicals, materials and food.

The programmes in LignoBiotech have always been ambitious and the spirit inspiring. The Symposium brings together cutting-edge research and dedicated researchers. We want to continue this tradition in Helsinki. LignoBiotech V provides an excellent forum for ca. 120 attendees from all over the globe to explore the most recent advances in the field. The Symposium will offer plenty of networking opportunities and discussions with the leadings scientists and researchers both from the industry and the academy.

The Symposium takes place in Katajanokka, just a few steps from the historical city center of Helsinki. In 1700's the Katajanokka island used to be a suberb of Helsinki. Now it is the harbour of many large cruise ferries and the Finnish icebreakers. This city district is well-known for its fine examples of Jugend Art Nouveau style architecture and landmarks including the Uspenski Cathedral. The early 1800 built neoclassic Senate Square forming the governmental center of Finland, the University of Helsinki main building and city center campus, the Kauppatori open Market Square, the Alvar Aalto designed Finnish headquarters of Stora Enso, and attractions like outdoor pool with sauna are all located nearby. The Suomenlinna Fortress and smaller islands at Helsinki coastline are easily accessible by ferries and boats leaving from the south harbour area.

We wish you a fruitful scientific meeting and a pleasant stay, hopefully you can also take some time to enjoy the beauty of Helsinki.

Kristiina Kruus, Taina Lundell, Kaisa Marjamaa, Mari Mäkinen
Symposium Organising Committee

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Conference organisation: *Confedent International, Helsinki, Finland*

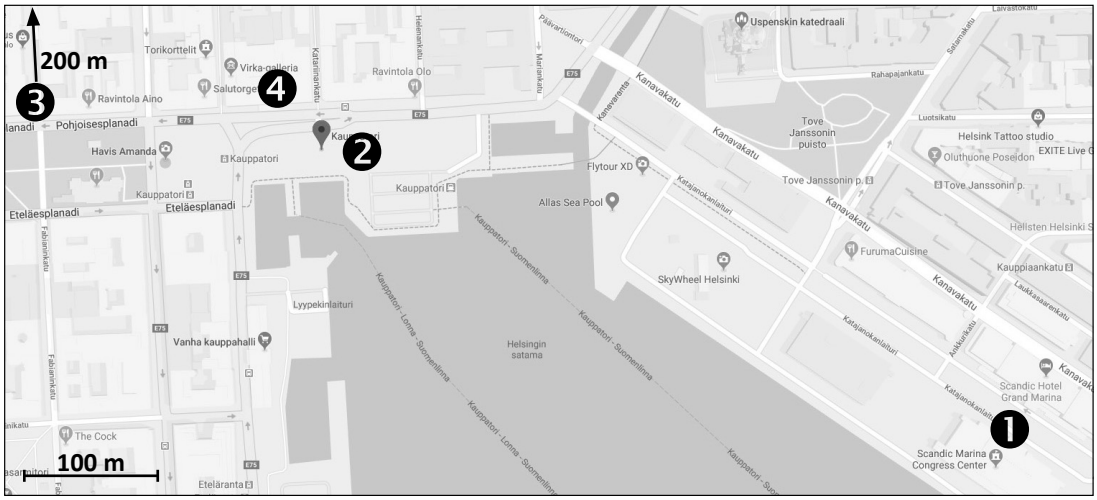
Abstract book: *PSWFolders Oy/Ltd, Helsinki, Finland*

SCHEDULE OF LIGNOBIOTECH 2018

Time	Wednesday August 29th	Thursday August 30th	Friday August 31st	Saturday September 1st
8:00-8:30		Registration		
8:30-9:00			Session 3	
9:00-11:00		Opening Session 1		Session 6
11:00-11:30		Coffee break	Coffee break	Coffee break
11:30-13:00		Session 1	Session 4	Session 7
13:00-14:00		LUNCH	LUNCH	Final remarks & closing 13:00 Farewell
14:00		Session 2	Session 5	
15:30	Registration 15:30-17:30			
16:00		Coffee break & Poster 1		
16:30			Coffee break & Poster 2	
17:30				
18:00-19:00	Reception 18:00-19:30			
19:00-			Dinner 19:00-23:00	

CONFERENCE SITE

The symposium sessions will take place during August 30th – September 1st at the **Scandic Marina Congress Centre (❶)**, street address: Katajanokanlaituri 6, 00160 Helsinki, located by the seaside in the Katajanokka district near to the **Kauppatori** (Salutorget) **(❷)** open Market Square of Helsinki City (see map below). Registration on Wednesday August 29th is arranged in the **University of Helsinki Main Building (❸)**, street address: Fabianinkatu 33. Helsinki City reception on Wednesday August 29th will take place in the **Helsinki City Hall (❹)**, address: Pohjoisesplanadi 11-13, located next to the Kauppatori Market Square. In this seaside old harbor and fish market district of Helsinki, you may find several cafes, bars and restaurants, shops, open air bath and sauna, other attractions, and enjoy the fresh breeze from the Gulf of Finland of the Baltic Sea.



Scandic Marina Congress Centre street view. Map and figure from Google

PROGRAMME

Day 1	Wednesday 29/08			
15:30 - 17:30	**Registration** University of Helsinki main building, Fabianinkatu 33			
18:00 - 19:30	**Helsinki City reception** Helsinki City Hall, Pohjoisesplanadi 11-13			
Day 2	Thursday 30/08	**Scandic Marina Congress Center, Fennia II** conference room		
8.00	**Registration**	Scandic Marina Congress Center, Katajanokanlaituri 6		
9.00	**Opening**	Kristiina Kruus, Taina Lundell		
Session 1. Lignocellulolytic enzymes			Time	Chair: Ligia Martins, Kaisa Marjamaa
9.10	**Plenary talk PT1**	Kiyohiko Igarashi	30+5 min	*Molecular mechanisms of cellulases*
9.45	Oral talk O1.1	Svein Horn	15+5	*Activation of LPMOs in a commercial cellulase cocktail*
10.05	Oral talk O1.2	Caroline Mosbech	15+5	*Natural catalytic function of CuGE glucuronoyl esterase*
10.25	Oral talk O1.3	Johan Larsbrink	15+5	*Structure-function studies of bacterial CE15 enzymes*
10.45	Oral talk O1.4	Simo Sarkanen	15+5	*Salicylate hydroxylase activity for pine wood pretreatment*
11.05	*Coffee break*		25 min	
Session 1. Lignocellulolytic enzymes			Time	Chair: Kiyohiko Igarashi, Kaisa Marjamaa
11.30	**Plenary talk PT2**	Angel T. Martínez	30+5 min	*Lignin-related enzymes*
12.05	Oral talk O1.5	Ligia Martins	15+5	*Evolving bacterial laccases and DyP-peroxidases for oxidation*
12.25	Oral talk O1.6	Susana Camarero	15+5	*Stable laccase engineered for valorization of kraft lignin*
12.45	Oral talk O1.7	Kristiina Hildén	15 min	*Selective cleavage of β-O-4 aryl ether bond by β-etherase*
13.00	*Lunch*		1 h	
Session 2. Microbial tansformation and omics				Chair: Angel T. Martínez, Mari Mäkinen
14.00	**Plenary talk PT3**	Igor Grigoriev	30+5 min	*Multi-omics of lignocellulose decomposition by fungi*
14.35	Oral talk O2.1	Ron de Vries	15+5	*An integrated network of transcriptional regulators*
14.55	Oral talk O2.2	Takehito Nakazawa	15+5	*Efficient gene disruption using the CRISPR/Cas9 system*
15.15	Oral talk O2.3	Ichiro Kamei	15 min	*Molecular breeding of white rot fungus Phlebia sp. MG-60*
15.45	**Poster session 1** *Coffee & refreshments*		2 h 15 min	
18.00	*free evening*			

Day 3	Friday 31/08	**Scandic Marina Congress Center, Fennia II** conference room		
Session 3. Lignocellulose analytics and structure				Chair: Emma Master, John Ralph
8.30	**Plenary talk PT4**	John Ralph	30+5 min	*Designing lignins for the biorefinery*
9.05	Oral talk O3.1	Gabriel Paes	15+5+5	*Towards universal features to understand lignocellulose recalcitrance?*
9.30	Oral talk O7.2	Aurelié Bichot	15+5	*Microwave pretreatment of corn and miscanthus stalks*
9.50	Oral talk O3.3	Aya Zoghlami	15+5	*Spatio-temporal imaging and quantification of lignocellulosic*
10.10	Oral talk O3.4	David Cannella	15+5	*Biophysical studies of cellulose oxidations - role of surface water*
10.30	Oral talk O3.5	Laurent Bleuze	15+5	*Remote sensing methods to monitor fiber crops processing*
10.50	*Coffee break*		40 min	
Session 4. Industrial examples of lignocellulose utilization				Chair: Kristiina Kruus, Kaisa Marjamaa
11.30	**Plenary talk PT5**	Anna Suurnäkki	30+5 min	*New bioproduct mill concept*
12.05	**Plenary talk PT6**	Tom Granström	30+5	*Chemicals and fuels from lignin stream of lignocellulose biorefinery*
12.40	**Plenary talk PT7**	Matti Heikkilä	30+5	*Enzymatic lignin modification and depolymerization*
13.15	*Lunch*		1 h	
Session 5. Lignocellulose biosynthesis and material activation				Chair: Susana Camarero, Taina Lundell
14.15	Oral talk O5.1	Kurt Fagerstedt	15+5 min	*Ray parenchymal cells actively contribute to lignification*
14.35	Oral talk O5.2	Takashi Watanabe	15+5	*Valorization of ligninocelluloses using microwave technology*
14.55	Oral talk O5.3	Barry Goodell	15+5	*Fungal-inspired catalytic lignocellulose deconstruction*
15.15	Oral talk O5.4	Kaisa Marjamaa	15+5	*Effect of enzyme family and structure on modification of wood fibres*
15.35	Oral talk O5.5	Mika Sipponen	15+5	*Cationic colloidal lignin particles in spatial immobilization of enzymes*
15.55	Oral talk O5.6	Elise Gerbin	15+5	*Lignin cross-linking on cellulose nanocrystal based films*
16.15	Oral talk O5.7	Valdeir Arantes	15 min	*Co-production of cellulose nanocrystals and industrial sugars*
16.30	**Poster session 2** *Coffee & refreshments*		1 h 30 min	
19:00-23:00	*Conference dinner*	Katajanokan kasino, Laivastonkatu 1		

Day 4	Saturday 01/09/2018	**Scandic Marina Congress Center, Fennia II** conference room		
Session 6. Chemicals and fuels from biomass				Chair: Lisbeth Olsson, David Canella
9.00	**Plenary talk PT8**	Lisbeth Olsson	30+5 min	*to be announced*
9.35	Oral talk O6.1	Krithika Ravi	15+5	*Towards bacterial valorization of low molecular weight lignin*
9.55	Oral talk O6.2	Aymerick Eydes	15+5	*Production of the platform chemical muconic acid in plant biomass*
10.15	Oral talk O6.3	Hans Mattila	15+5	*Bioethanol from untreated waste lignocellulose materials*
10.35	Oral talk O6.4	María Hijosa Valsero	15+5	*Simplified method for biobutanol production from corn stover*
10.55	*Coffee break*		30 min	
Session 7. Bioconversion and treatment of lignocelluloses				Chair: to be announced
11.25	Oral talk O7.1	Mark Blenner	15+5 min	*Pathways for conversion of lignin-derived aromatics to lipids*
11.45	Oral talk O3.2	Hiroshi Nishimura	15+5	*Fractionation and structural analysis of enzyme-treated*
12.05	Oral talk O7.4	Eduardo Diaz	15+5	*Engineering bacterial biocatalysts for bioconversion of lignin-derived*
12.25	Oral talk O7.5	Taina Lundell	15 min	*Wood-decay fungi for sustainable bioeconomy on lignocelluloses*
12.40	*General Discussion & Symposium Closing*		20 min	Kristiina Kruus, Taina Lundell, Emma Master
	Announcement of the next symposium for 2020			
13.00	*Farewell*			

ABSTRACTS

PT1

Molecular mechanisms of cellulases

Kiyohiko Igarashi[1,2]

[1]Graduate School of Agricultural and Life Sciences, the University of Tokyo, [2]VTT Technical Research Centre of Finland

Cellulose is a major component of plant cell wall and the most abundant biomass on earth. Efficient degradation of cellulose makes it possible to produce fuels and chemicals from plant resources for the achievement of Bioeconomy, although biochemical conversion of cellulose by cellulase is quite slow and the reaction becomes a bottleneck of the process. We recently reported the real-time visualization of crystalline cellulose degradation by individual cellulase molecules using a high-speed atomic force microscopy, having sub-second time resolution and nanometer space resolution. I will summarize possible molecular mechanisms of the processive enzymes and the natural degradation of crystalline cellulose in addition to the recent neutron crystallography to clarify the detailed hydrolytic mechanisms of inverting cellulases.

O1.1

Activation of LPMOs in a commercial cellulase cocktail by controlled addition of hydrogen peroxide

Gerdt Müller[1], Piotr Chylenski[1], Bastien Bissaro[1], Line Degn Hansen[1], Aniko Varnai[1], Vincent Eijsink[1], **Svein Jarle Horn**[1]

[1]Norwegian University of Life Sciences (NMBU)

The oxidative enzymes called Lytic Polysaccharide Monooxygenases (LPMOs) have improved the efficiency of current commercial cellulase cocktails for saccharification of lignocellulosic biomass. Building on the recent discovery that H_2O_2, rather than O_2, is the co-substrate of LPMOs, we show how cellulose degradation by an LPMO-containing commercial cellulase cocktail can be controlled and enhanced by adding H_2O_2. Controlled pumping of H_2O_2 into a reactor results in high LPMO rates in the absence of molecular oxygen with only sub-stoichiometric consumption of reductant. We report saccharification rates and yields for a model substrate (Avicel) and industrial lignocellulosic substrates that, at low H_2O_2 feed rates, are higher than those seen under standard aerobic conditions. In an industrial setting, addition of the liquid H_2O_2 to a reactor is easier and cheaper than supplying molecular oxygen. Additionally, the use of H_2O_2 to activate LPMOs makes it possible to design novel combined saccharification and fermentation processes.

O1.2

Natural catalytic function of CuGE glucuronoyl esterase in hydrolysis of genuine lignin-carbohydrate complexes from birch

Caroline Mosbech[1], Jesper Holck[1], Anne S. Meyer[1], Jane Wittrup Agger[1]

[1]*Center For Bioprocess Engineering, Department Of Chemical And Biochemical Engineering, Technical University Of Denmark.*

Cross links in plant cell walls, such as lignin-carbohydrate complexes (LCCs) create challenges for selective separation and isolation of lignin in enzymatic conversion of plant biomass. Glucuronoyl esterases (CE15 family) are presumed to enable targeted cleavage of ester linkages in LCCs, particularly those linking lignin and glucuronoyl residues in hardwood xylan. We here present for the first time a detailed product profile of aldouronic acids released from birchwood lignin by a glucuronoyl esterase from the white-rot fungus Cerrena unicolor (CuGE). CuGE acts synergistically with GH10 endo-xylanase resulting in significantly increased product release compared to the action of endo-xylanase alone. The data verify the enzyme's unique ability to catalyze removal of all glucuronoxylan associated with lignin, a function we suggest as important for the fungal organism's ability to effectively utilize all available carbohydrates in lignocellulosic substrates.

O1.3

Structure-function studies of bacterial CE15 enzymes

Scott Mazurkewich[1], Jenny Arnling Bååth[1], Rasmus Meland Knudsen[2], Jens-Christian Navarro Poulsen[2], Lisbeth Olsson[1], Leila Lo Leggio[2], **Johan Larsbrink**[1]

[1]*Wallenberg Wood Science Center, Division of Industrial Biotechnology, Department of Biology and Biological Engineering, Chalmers University Of Technology,* [2]*Department of Chemistry, HC Ørsteds Institutet, Copenhagen University*

Glucuronoyl esterases (GEs) are enzymes proposed to cleave covalent ester bonds found between glucuronoxylan and lignin. As such, these enzymes potentially play a crucial role in reducing the recalcitrance of complex plant biomass, by facilitating substrate access for other carbohydrate-active enzymes. GEs are found in Carbohydrate Esterase family 15 (CE15), which is underexplored both regarding biochemical characterization of its members and regarding structural determinations. Studies of bacterial CE15 enzymes are especially lacking, with only two partially characterized enzymes to date. Here, we report detailed biochemical characterization of eleven bacterial enzymes encoded by four species, as well as three new protein structures. In addition, transcriptional analyses reveal how different CE15 enzymes are regulated by different biological queues in a given bacterium, and how supplementation of commercial enzyme cocktails with GEs can dramatically boost the release of monosaccharides during biomass hydrolysis.

O1.4

Salicylate Hydroxylase Activity for Effective Pine Wood Pretreatment

Yi-ru Chen[1], **Simo Sarkanen**[1], Yun-Yan Wang[2]

[1]University of Minnesota, [2]University of Tennessee

Versatile peroxidase has been reported to enhance cellulase-catalyzed saccharification of ball-milled corn stover by 14% (Biotechnol. Biofuels 2017; 10:218). It would be desirable to elucidate whether substrate delignification was responsible for the effect, or whether there was competition between cellulase and versatile peroxidase in adsorbing onto lignin domains. On the other hand, pretreatment of ball-milled pine wood with Pseudomonas salicylate hydroxylase can engender a 31% increase in the cellulase-catalyzed saccharification yield obtained at pH 6.3 (U.S. Patent 2017, No. 9,796,993). In open solution, salicylate hydroxylase has a 2-fold effect on dissolved native lignin. The radius of gyration of the macromolecular substrate rapidly decreases as a result of cleavage. Concomitantly, the molecular weight increases through a process caused by (non-productive) enzyme-mediated association. Only in the absence of the salicylate-hydroxylase cofactor, NADH, can an initial reduction in lignin molecular weight be seen, along with a consistent fall in the radius of gyration.

PT2

The oxidative biodegradation of lignin as seen from the "enzyme side"

Verónica Sáez-Jiménez[1,2]*, Jorge Rencoret[3]*, Ana Gutiérrez[3], Francisco J. Ruiz-Dueñas[2], **Angel T. Martínez**[2]

[1]Chalmers University, Gothenburg, Sweden; [2]CIB, CSIC, Madrid, Spain; [3]IRNAS, CSIC, Seville, Spain
**these two authors equally contributed to the work*

Methodological limitations difficult estimation of lignin oxidation rates by ligninolytic peroxidases. Here, lignin oxidation was seen from the "enzyme side" by estimating the reduction rates of H2O2-activated peroxidase compounds I and II, using rapid spectrophotometry. Removal of Trp164 in versatile peroxidase resulted in 4-100 fold slower rate-limiting compound-II reduction by water-soluble sulfonated lignins. Moreover, when methylated lignosulfonates were used, negligible transfer was found, confirming that residual reduction by native lignin was due to its phenolic moiety. In agreement with the transient-state kinetic data, low or null structural modification of lignin was found (by NMR) during steady-state treatment with the W164S variant compared with native VP. Similar results were obtained with lignin peroxidase. Therefore, we demonstrate for the first time that the surface tryptophan conserved in most LiPs and VPs is strictly required for oxidation of the nonphenolic moiety, which represents the major and more recalcitrant part of the lignin polymer.

O1.5

Evolving bacterial laccases and DyP-peroxidases for oxidation of lignin-related phenolics

Lígia O Martins[1], Vânia Brissos[1], Diogo Silva[1], Luís Vicente[1], Zuleica Duarte[1], AC Sousa[2], MP Robalo[2]

[1]ITQB Nova, [2]ISEL, IST-UL

Bacterial enzymatic systems for lignocellulose degradation are less explored as compared with those of fungal origin but may provide a rich source of new ligninolytic and auxiliary enzymes given the breath of bacterial enzymes and pathways that functionalize or degrade aromatics. Furthermore, they hold a good potential considering the easiness of gene cloning, protein production and the high number of molecular tools available for enzyme engineering. We have used directed evolution through random mutagenesis by error-prone PCR or DNA-shuffling, of bacterial laccase and DyPs-genes followed by high-throughput screening to improve the efficiency of enzymes for lignin-related phenolic compounds. This approach has led to the identification of bacterial laccase-like (McoA) and DyP peroxidase (PpDyP) variants featuring up to 100-fold enhanced catalytic efficiency for phenolic lignin-related substrates. These studies opened perspectives for further evolution of these enzymes for improved properties that are major limiting factors for their industrial application.

O1.6

A stable laccase engineered in the lab as biocatalyst for the valorization of kraft lignin

Isabel Pardo[1,2], Felipe de Salas[1], Pablo Aza[1], David Rodrigues-Escribano[1], **Susana Camarero**[1]

[1]Centro de Investigaciones Biológicas, CSIC, Madrid, Spain, [2]National Renewable Energy Laboratory, Golden, CO

A new fungal laccase variant notably stable to high temperature, extreme pH and the presence of organic co-solvents, was engineered by structure-guided recombination of two high redox potential laccases. Specifically, the second cupredoxin domain of PM1 basidiomycete laccase evolved in yeast1 was exchanged with that of evolved Pycnoporus cinnabarinus laccase2, thus introducing a pool of neutral mutations without jeopardizing protein folding or enzyme function. The new variant expressed in Saccharomyces cerevisiae showed improved catalytic efficiency oxidizing phenolic compounds, optimal activity at 65–70 ºC and substantially superior half-life at high temperatures. This highly stable laccase was assayed as biocatalyst for the modification of Kraft lignin. As compared with the parent type, the new variant enabled superior modification of lignin at high temperature and short incubation times, as revealed by SEC analyses (with no addition of redox mediators). The engineered laccase is a promising biocatalyst for valorizing industrially relevant lignin streams.

O1.7

Selective cleavage of B-O-4 aryl ether bond by B-etherase of the white-rot fungus Dichomitus squalens

Mila Marinovíc[1], Paula Nousiainen[2], Adiphol Dilokpimol[3], Jussi Kontro[2], Robin Moore[2], Jussi Sipilä[2], Ronald de Vries[3], Miia Mäkelä[1], **Kristiina Hildén**[1]

[1]Fungal Genetics and Biotechnology group, Department of Microbiology, University of Helsinki, [2]Department of Chemistry, Faculty of Sciences, University of Helsinki, [3]Fungal Physiology, Westerdijk Fungal Biodiversity Institute-KNAW & Fungal Molecular Physiology, Utrecht University

Enzymatic catalysis with selective cleavage of lignin bonds provides a sustainable option for lignin valorization. We present the first functionally characterized fungal intracellular β-etherase, Ds-GST1, from the wood-degrading white-rot basidiomycete Dichomitus squalens. Ds-GST1 belongs to the glutathione-S-transferase superfamily and selectively cleaves the β-O-4 aryl ether bond of a dimeric lignin model compound in a glutathione-dependent reaction. Ds-GST1 also demonstrates activity on polymeric synthetic lignin fractions, shown by a decrease in molecular weight distribution of the laccase-oxidized guaiacyl-DHP. In addition to a possible role of Ds-GST1 in intracellular catabolism of lignin-derived aromatic compounds, the cleavage of the most abundant linkages in lignin under mild reaction conditions makes this biocatalyst an attractive green alternative in biotechnological applications.

PT3

Multi-omics of lignocellulose decomposition by fungi

Igor Grigoriev[1]

[1]US Department of Energy Joint Genome Institute, [2]Department of Plant and Microbial Biology, University of California Berkeley

The first sequenced genomes of wood decay fungi Phanerochaette crysosporium and Postia placenta revealed both enzymes and Fenton chemistry involved in decomposition of lignocellulose. A broader sampling of Agaricomycetes containing majority of wood decay fungi helped reconstruct their evolutionary history and catalogs of lignocellulolitic enzymes (CAZymes), rejected the white-rot/brown-rot dichotomy, and built a critical mass of data for machine learning with over 250 Agaricomycete genomes in JGI MycoCosm (jgi.doe.gov/fungi). Further genome explorations of the Fungal Tree of Life found not only the largest expansion of CAZymes in anaerobic gut fungi from Neocalimastigomycota but also cellulosomes encoded in their genomes. In addition to reference genomes, transcriptomics, proteomics, and metabolomics data enrich the framework for further multi-omics exploration of mechanism of action, substrate specificity, and interactions with other microbes.

O2.1

An integrated network of transcriptional regulators mediates lignocellulose conversion in filamentous fungi

Ronald de Vries[1]

[1]*Westerdijk Fungal Biodiversity Institute*

Lignocellulose is not only an important substrate for the biobased economy, but also the predominant carbon source for many fungi. Recent studies revealed significant differences in fungal approaches for lignocellulose conversion. While some of these are obvious from the genome content of individual fungi, many differences appear to originate at the regulatory level. For instance, despite high similarity in genome content, Aspergillus species produce highly diverse enzyme sets when exposed to the same lignocellulolytic substrate.
A better understanding of the regulatory mechanisms that control the production of lignocellulolytic enzymes is essential for rational improvements of commercial enzyme cocktails. Such understanding will also provide insights into the temporal and substrate specific production of individual enzymes.
In this presentation recent insight into these regulatory systems will be revealed and examples of parallel evolution, hierarchical order and interaction of regulators will be provided, focusing on ascomycete fungi.

O2.2

Efficient gene disruption using the CRISPR/Cas9 system in white-rot fungi Pleurotus ostreatus and Ceriporiopsis subvermispora

Chikako Inoue[1], **Takehito Nakazawa**[1], Dong Xuan Nguyen[1], Ryota Morimoto[1], Keishi Osakabe[2], Yoichi Honda[1]

[1]*Kyoto University,* [2]*Tokushima University*

Genetics study is important to identify factors that affect the ligninolytic activity in white-rot fungi. However, it is almost impossible to conduct gene targeting experiments in white-rot fungi except Pleurotus ostreatus and Schizophyllum commune. Genome editing is considered one of promising methods that can introduce gene mutations in various fungi. Recently, a CRISPR/Cas9 system was established in the model mushroom Coprinopsis cinerea (Sugano et al., 2017). We here successfully established genome editing in P. ostreatus and the selective white-rot fungus Ceriporiposis subvermispora according to the procedure described by Sugano et al. (2017). Then, we generated gene disruptants of two fungi defective in the ligninolytic activity. In C. subvermispora, a transient transformation was performed to establish genome editing, and it was suggested that about 30% transformants lost the Cas9/gRNA-expression plasmid during repeated transfers, indicating that multi-gene mutations can be generated by repeating the same procedure.

O2.3

Molecular breeding of white rot fungus Phlebia sp. MG-60 to convert lignocellulose materials

Ichiro Kamei[1], Taichi Motoda[1], Taku Tsuyama[1]

[1]*University of Miyazaki*

Ethanologenic white-rot fungus Phlebia sp. MG-60-P2 produces ethanol directly from several lignocelluloses. In this study, we evaluated the effect of knockout and RNAi-mediated silencing of the pyruvate decarboxylase gene of Phlebia sp. MG-60-P2 (MGpdc1). Then the co-transformations of lactate dehydrogenase gene (ldhA) expression vector (pLDH) or RNAi construct of b²-glucosidase gene (pMGBGL-RNAi) with RNAi construct of MGpdc1 (pMGPDC-RNAi) are also tested in carbohydrates conversion. The MGpdc1 knockout and RNAi lines showed a variety of suppression levels of ethanol production and MGpdc1 expression, and this variation led to different metabolic fluxes, resulting in rapid accumulation of xylitol from xylose and of glucose from cellulose. Co-transformation of pLDH and pMGPDC-RNAi shows the slight accumulation of lactic acid from glucose. Co-transformation of pMGBGL-RNAi and pMGPDC-RNAi shows the accumulation of cellobiose from cellulose. The physiological role of ethanol fermentation in wood rotting will be also discussed.

PT4

Designing Lignins for the Biorefinery

John Ralph[1], José Carlos del Rio[2], Jorge Rencoret[2], Shawn Mansfield[3], Yaseen Mottiar[3], John Grabber[4], Hoon Kim[1], Steven Karlen[1], Rebecca Smith[1], Yanding Li[1], Troy Runge[1], Fachuang Lu[5], Yuki Tobimatsu[6], Shinya Kajita[8], Wout Boerjan[7]

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As lignin is a polymer formed from its monomer radicals by purely chemical radical coupling reactions, the breadth of options for 'designing' the composition and structure of lignins is unparalleled. Caffeyl alcohol-derived lignin polymers represent one class of 'ideal lignins'. New phenolic monomers can be introduced into the polymer. Inspired by Nature's incorporation of the non-lignin-pathway flavonoid, tricin, into monocot lignins, and hydroxystilbenes in palm endocarp lignins, researchers can now contemplate plants in which the lignins incorporate valuable components that can be subsequently retrieved from the 'waste' streams. Moreover, lignins have now been successfully 'redesigned' to contain readily chemically cleavable ester bonds in the polymer backbone, facilitating improved industrial processing – for example, chemical pulping, or pretreatment options for the saccharification of wall polysaccharides to sugars for liquid biofuels production. We suspect that we are now entering a reinvigoration period for lignin research aimed at its manipulation for improved utilization.

O3.1

Towards universal features to understand lignocellulose recalcitrance?

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Recalcitrance of lignocellulose is caused by its structural and chemical complexity. Predicting its transformation, in particular enzymatic hydrolysis into monosaccharides, is very challenging and also critical for building versatile thus viable biorefineries.
Until now, many different features controlling recalcitrance have been highlighted but many studies are controversy and measurement of most of these features requires time-consuming lab analysis. We have investigated new spectral features and have tested them on various biomass species and pretreatments. Correlations with saccharification was found to be very strong. We discuss the potential of these easily-measured features and the possibility of finding universal features (or not) of lignoellulose recalcitrance.

O3.2

Fractionation and structural analysis of enzyme-treated lignin-carbohydrate complexes

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Lignin is linked to hemicellulose by covalent bindings and form Lignin-Carbohydrate Complexes (LCCs) in wood cell walls. However, the frequency of binding is lower than that in the polymer main chain. Therefore, preparation of concentrated and purified LCC is essential for the entire structural analysis. In this study, we aimed to concentrate LC bindings by purifying LCC from natural wood by combining two steps of enzymatic treatments. We prepared LCC fraction by the reaction of polysaccharide-degrading enzymes and ligninolytic enzymes followed by the purification. We characterized LC bonds by 2D-NMR and elucidate some specific linkages in combination with long-range correlated HMBC and TOCSY-HSQC NMR.

O3.3

Spatio-temporal imaging and quantification of lignocellulosic biomass deconstruction during enzymatic hydrolysis

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Lignocellulosic biomass (LB) is a renewable resource from plants used as an alternative to fossil resources [1]. However, LB is recalcitrant to enzymatic deconstruction due to its chemical composition and its structural complexity [2].

Different chemical and physical features have been proposed to explain LB recalcitrance and to predict its deconstruction [4] However none of them seems to be universal but rather specific to biomass species and pretreatment. One key progress is to achieve a better understanding of the evolution of the 3D architecture of LB during the enzymatic hydrolysis through 4D imaging.

Our project aims at setting up a novel approach to quantify the dynamics of enzymatic deconstruction of LB using 4D fluorescence confocal microscopy to identify the key structural features correlated with enzymatic hydrolysis.

O3.4

Biophysical studies of cellulose oxidations and the role of surface water binding during enzymatic hydrolysis

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For industrial biomass transformation the enzymatic hydrolysis of cellulose occurs at high dry matter (HDM), a mandatory condition that often causes a decrease of conversion into the final products. The main drawback of HDM is the reduction of water activity and availability for the solvation of progressively increasing concentration of hydrolyzed glucose molecules that causes an inhibition of hydrolytic enzymes (CBHs, EG, Beta-glucosidases). Cellulose surface oxidation changes the interactions with the surrounding water molecules with the respect to the not oxidized regions of the cellulose. The carboxyl groups introduced with the oxidation bring a net negative charge which might increases the solubility of small part of cellulose by mostly establishing new polar bonds (hydrogen bonds). Thus the enzymatic oxidation performed by the lytic polysaccharide monooxygenases (LPMO) could favor the binding of water to the cellulose surface, essential for the subsequent enzymatic hydrolysis.

O3.5

Remote sensing methods to monitor fiber crops processing

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Novel lignocellulosic biomass valorisations have emerged as plant fibres (hemp, flax) intended to replace synthetic fibers in composites materials. Fiber quality management remains critical and one major challenge concerns dew-retting which is a stem selective biodegradation achieved on the soil facilitating fibre extraction preserving fibres mechanical properties and quality. This study aims at deeper understanding of the hemp stems dynamics during dew-retting because most knowledge remains empirical.

Remote sensing methods such as infrared spectroscopy and colorimetry were used as non-destructive, fast and low cost methods as alternatives to destructive and time-consuming wet chemical analysis. These approaches allowed identifying new fast monitoring indicators of the stem retting dynamic enabling the improvement of fiber quality during processing. Both stem surface color (CIELab) evolutions and surface chemical composition changes measured by attenuated total reflection Fourier transform infrared (ATR-FTIR) revealed the progressive microbial colonization and degradation in agreement with wet chemistry and microscopic analysis.

PT5

New bioproduct mill concept

Anna Suurnäkki[1]

[1]*Metsä Fibre Oy*

Modern kraft pulp mill is a highly efficient wood biorefinery. Wood fibres are processed to kraft pulp while part of the wood components is traditionally converted to bioenergy and biochemicals turpentine and tall oil. Many components in the streams of a kraft mill could, however, be potential as starting materials for other added-value wood-based products. The first implementation of the new bioproduct concept, with a modern kraft pulp mill as the core, is Metsä Group's Bioproduct mill in Äänekoski inaugurated in 2017. The leading idea of this mill is to convert 100% of wood and side streams to biomaterials, biochemicals and bioenergy by sustainable and resource efficient processes without fossil fuel. Currently, already several new bioproduct concepts have been realized in demonstration or full scale in the mill. There might be more in the future as the mill has been planned to support gradual implementation of new feasible bioproduct concepts.

PT6

Chemicals and fuels from lignin stream of lignocellulose biorefinery

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[1]*St1 Renewable Energy Oy*

St1 Cellunolix® process is producing bioethanol as a main product, but also lignin, turpentine, furfural, concentrated fermentation rank and C02 from softwood sawdust. The process is based on steam explosion, enzymatic hydrolysis, filtration, fermentation and distillation unit operations. In St1 Kajaani lignocellulose biorefinery plant the annual production volume of lignin side product is approximately double of that to bioethanol. Therefore, the revenue from lignin has a significant contribution to the cost effectiveness of the entire process. In solid form lignin can be used for material, biofuel or animal feed applications. Lignin can be depolymerized and liquified enzymatically, catalytically and thermochemically allowing different commodity chemicals, gaseous and biochar products. Liquified lignin stream can also be upgraded and co-fed into oil refinery streams or potentially converted into jet fuel. From the industrial point of view, it is utmost important to create profitable business from lignin to promote the transformation from fossil energy resources to carbon free renewable energy production.

PT7

Enzymatic Lignin Modification and Depolymerization

Matti Heikkilä[1]

[1]*MetGen Oy*

Main hurdles of lignin valorization are its diverse chemical composition, recalcitrance, and poor solubility due to high molecular weight and branched structure. Oxidative enzymes have long been proposed as a promising tool in lignin depolymerization. Their application was limited to ambient pH, where lignin is poorly soluble in water. MetGen developed and brought to market several lignin oxidizing enzymes, including an extremely alkaline lignin oxidase MetZyme® PURECO™ that functions at pH 11 and elevated temperatures, addressing lignin at its water-soluble state. Under these conditions, not only the molecular weight but also solubility in water and solvents as well as water dispersion properties of lignin were altered. Importantly, solvent-free soluble lignin fragmentation allowed for robust industrial membrane separation technologies to be applicable for product fractionation. The enzymatic technologies are ready for licensing and integration to an industrial scale biorefinery is in progress through EU and BBI JU Flagship Project SWEETWOODS.

O5.1

Ray parenchymal cells actively contribute to lignification of tracheids in developing xylem of Norway spruce

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[1]*University of Helsinki,* [2]*Umeå Plant Science Centre,* [3]*Natural Resources Institute Finland, LUKE*

A transcriptomic study was conducted to find out whether parenchymal ray cells participate in the biosynthesis of monolignols in Norway spruce trunks by supplying monolignols. Laser scanning microdissection was used to cut out parenchymal ray cells and upright tracheids from tangential cryosections of xylem. RNA was isolated from ray cells, upright tracheids and whole sections. Transcriptome analysis revealed that in both developing tracheids and in developing ray cells genes encoding cell wall biogenesis-related enzymes were highly expressed. Especially, most of the shikimate and monolignol biosynthesis pathway genes were equally expressed in both cell types. However, 1073 differentially expressed genes were detected, with transcripts of 541 genes more abundant and 532 genes less abundant in ray cells than in tracheids. The results indicate that the biosynthetic route for monolignols is active both in upright tracheids and in parenchymal ray cells, and hence they can participate in the lignification of xylem tracheids.

O5.2

Approaches for valorization of ligninocelluloses using microwave technology and lignin-binding catalysts

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Lignin is the sole large-volume renewable feedstock composed of an aromatic skeleton. Highly selective degradation of lignin is pivotal for lignocellulosic biorefinery. We studied production of lignin-derived aromatic chemicals for functional polymers using microwave reactions. In reactions of wood particles with CuO and H_2O_2, MW heating produced vanillin and vanillic acid in yields nearly three times higher than those produced by conventional heating. Experiments with a cavity resonator revealed that the reactions were accelerated by electric and magnetic fields with a slightly more prominent effect of electric field. We identified lignin-binding peptides and have been developing ligninolytic catalysts having an increased affinity to the lignin. The catalytic properties toward lignocelluloses are reported.

O5.3

Catalytic Pretreatment and Cellulase Cocktail Mimics Brown Rot for Potential Biorefinery Applications

Steven Tabor[1], Lourdes Orjeula[2], David Contreras[3], Gry Alfredsen[4], Jody Jellison[5], Scott Renneckar[6], Nicole (Niki) Labbe[7], **Barry Goodell**[1]

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A “chelator-mediated Fenton” (CMF) treatment was used with an enzymatic cocktail to study the mechanisms of brown rot fungi, but also to explore improved treatment processes for bioprocessing of woody biomass. Our data suggest that the CMF mechanism is highly efficient in overcoming the lignin recalcitrance barrier to solubilize wood. Up to 4 pulses of CMF treatment were able to solubilize a majority of both the lignin and cellulose of wood at room temperature, using a hydrogen peroxide concentration of 1%. Using a single pulse of the CMF system as a pretreatment allowed more wood residue to be retained, and enzymatic action on this pretreated wood was enhanced compared to control wood. In separate experiments, significantly greater solubilization of both sugars and lignin occurred when a single-pulse CMF pretreatment was used prior to enzymatic action than by enzymatic action alone on unmodified wood.

O5.4

Effect of enzyme family and structure on modification of wood fibres at high consistency

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Cellulose is an interesting option for future material applications in textiles, composites, and in packaging. Enzymes are specific tools for modification of cellulosic pulps for improved material properties. The action of enzymes at high pulp consistency, desired in industry, has been rarely studied. The role of enzyme family and structure in fibre modification at high consistency was assesed here using monocomponent endoglucanases from three structurally different glycoside hydrolase families (5, 7 and 45) with and without carbohydrate binding module (CBM). Enzyme treatment at high consistency (20 % w/w) was found to enhance enzyme activity more than 6-fold compared to fibre treatment at low dry matter consistency (1 % w/w). The glycoside hydrolase family 45 endoglucanase was found to be most specific in acting on pulp cellulose whereas the family 5 and 7 endoglucanases had activity on pulp hemicelluloses as well. CBM did not improve enzyme action at high pulp consistency.

O5.5

Cationic colloidal lignin particles in spatial immobilization of enzymes for aqueous ester synthesis

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Enzymatic esterification is imparted by the predominant hydrolysis reaction in aqueous media. We found that this barrier can be overcome by immobilization of hydrolases in spatially confined compartments formed from cationic colloidal lignin particles. The immobilized enzymes were used to synthesize butyl butyrate in yields exceeding 80% in repeated 24 h aqueous-organic biphasic reactions. The catalysts retained a majority of their synthetic activity even in the presence of 90% volume fraction of water, outcompeting the commercial benchmark enzyme Candida antarctica lipase B immobilized in acrylic resin in this regard.

O5.6

Lignin cross-linking on cellulose nanocrystal based films by hydroxyl radicals

Elise Gerbin[1,2], Emmanuel Bertrand[3], David Crônier[1], Betty Cottyn-Boitte[2], Paul Henri Ducrot[2], Yves Michel Frapart[4], Stéphanie Baumberger[2], Bernard Kurek[1], Véronique Aguié-Béghin[1]

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The uses of lignins and nanocelluloses is of utmost interest to design new functionalities for composites materials, in a wide range of applications. For such purpose, Fenton reaction has been recently used to graft lignin onto nanocellulose surfaces to form films with interesting properties [2]. In order to better control the reactivity of the highly oxidative species generated during Fenton reaction, a new pathway using of Cellobiose DeHydrogenase (CDH) to generate the hydrogen peroxide (H_2O_2) was developed.
In this context, two systems where H_2O_2 is added or generated by CDH, were studied by analyses of oxidised products and radicals formed during the reactions using LC-MS and electron paramagnetic resonance (EPR). In parallel, the impact of hydroxyl radicals was evaluated on the physical and chemical properties of films obtained by these two systems. The challenge is to control functional properties of films as transparency, UV-blocker, water resistance and anti-oxidant properties.

O5.7

Co-production of cellulose nanocrystals and industrial sugars at high concentration via two-stage enzymatic hydrolysis process

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The isolation of cellulose nanocrystals by controlled enzymatic hydrolysis of cellulose with cellulases has many advantages over the traditional acid hydrolysis: no need for expensive acid-resistant equipment, fewer process steps, low water requirement, and the only products in the water stream at the end of the process are sugar molecules. However, the concentration of sugars in this water stream is very low, since the hydrolysis for isolation of CNC is typically carried out at low consistency due to the well-known declining in cellulase's efficiency at high consistency. In this presentation, we will present a two-stage hydrolysis process approach developed to allow carrying enzymatic hydrolysis of cellulose at high concentration, consequently making it possible to recovery of the solubilized sugar at high concentration in addition to isolation of CNC. This presentation will also discuss the viability of this two-stage approach to decrease the required cellulase dosage for isolation of the nanoparticles.

PT8

Title TBC

Lisbeth Olsson

O6.1

Towards bacterial valorization of low molecular weight lignin and lignin-related compounds

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Lignin is a potential source of renewable chemicals, but current exploitation in the industry for this purpose is still low. Bacterial conversion of lignin is one of the possible routes to valorize lignin in a biorefinery setting. In this contribution, results form a screening and characterization study on the bacterial species which can degrade/metabolize lignin-related compounds, will be presented (Part of a larger project at Lund University, aiming towards the production of high value chemicals from lignin. http://www.lignin.lu.se/). An investigation was made on the growth of different bacterial species with thermochemically depolymerized softwood-based Kraft lignin (Indulin AT) as a carbon source. It was found that Rhodococcus opacus DSM 1069 and Pseudomonas fluorescens DSM 50090 can consume/ breakdown the lower (<0.4 kDa) and higher (1 – 10 kDa) molecular weight lignin respectively. Further experimental findings on the subfractions of depolymerized lignin will be discussed.

O6.2

Production of the platform chemical muconic acid in plant biomass

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Muconic acid (MA) is used for the production of chemicals such as adipic acid, terephthalic acid, and caprolactam. Synthesis of these polymer precursors utilizes petroleum-derived chemicals, and the development of alternative strategies for bio-based production of MA has garnered significant interest. Plants represent advantageous hosts for engineered metabolic pathways towards the manufacturing of chemicals. We demonstrate that plants can be used for the bio-manufacturing of MA. In particular, co-expression of bacterial salicylate hydroxylase (NahG), catechol 1,2-dioxygenase (CatA), salicylate synthase (Irp9), and feedback-resistant 3-deoxy-D-arabino-heptulosonate synthase (AroG) resulted in the conversion of the shikimate-derived salicylic acid pool into MA. This value-added co-product was easily recovered after biomass pretreatment. The elucidation and implementation in bioenergy crops of MA biosynthetic routes that divert phenylpropanoid pathway intermediates away from lignin biosynthesis will be presented. These engineering strategies combine in plant biomass the production of value-added chemicals with low-recalcitrance traits towards sustainable development of biorefineries.

O6.3

Bioethanol from untreated waste lignocellulose materials - Transcriptomics of Phlebia radiata under fermentative conditions

Hans Mattila[1], Mari Mäkinen[1], Anna Hartikainen[1], Taina Lundell[1]

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Renewable lignocellulose waste materials such as straw and sawdust are abundant, underused and sustainable sources for production of different biocompounds to replace the thinning fossil fuels resources. We have previously stated that several untreated lignocelluloses can be bioconverted into bioethanol in a simultaneous saccharification and fermentation (SSF) process by a wood-degrading white-rot fungus Phlebia radiata. To better understand the SSF process, P. radiata was cultivated either under aerobic or fermentative conditions and the transcriptomes and ethanol fermentation related metabolite profiles were compared.

P. radiata is capable of producing ethanol from untreated wood-waste in microaerophilic conditions while only trace quantities of side products such as acetate and glycerol are detected. The P. radiata transcriptome is very different during fermentation in comparison to aerobic conditions especially concerning cellulose, hemicellulose and pectin degradation and catabolism. Comparison to other P. radiata transcriptomes has also revealed several co-regulated gene clusters within the core metabolic pathways.

O6.4

A simplified method for biobutanol production from corn stover

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Corn stover has been extensively studied as a feedstock for bioethanol production, and industrial technologies have been developed for this purpose. However, its transformation into biobutanol has only been assessed from a theoretical perspective or at laboratory-scale. Biobutanol is obtained by ABE bacteria which transform simple sugars into solvents. During the pretreatment of corn stover to release monosaccharides, inhibitory compounds that hinder ABE fermentation are generated. Therefore, detoxification steps prior to fermentation are generally needed.
A simplified method is proposed for the production of butanol from corn stover by acid hydrolysis with 0.9% H2SO4 during short times to reduce inhibitors generation, followed by enzymatic hydrolysis. Bacterial strain screening enables the production of about 6 g/L butanol from the hydrolysate without detoxification. Currently, hydrolysis and fermentation parameters are being improved to increase butanol concentrations.

O7.1

Discovery and engineering of pathways for conversion of lignin-derived aromatics to lipids by Cutaneotrichosporon oleaginosus

Allison Yaguchi[1], Michael Spagnuolo[1], Nicole Franaszek[1], Alana Robinson[1], Erin Mihealsick[1], **Mark Blenner**[1]

[1]*Clemson University*

Cutaneotrichosporon oleaginosus, previously known as Cryptococcus curvatus, is a non-model oleaginous yeast that is known for its ability to metabolize many alternative sugars, including xylose, and tolerate toxic lignocellulosic hydrolysate inhibitors such as 5-hydroxymethylfurfural and furfural. We discovered C. oleaginosus also tolerates and fully metabolizes lignin-derived aromatics including phenol, 4-hydroxybenzoic acid, p-coumarate, and resorcinol as sole carbon sources, as well as in co-utilization with glucose and xylose. We demonstrated lipid accumulation to over 69% of biomass by weight. RNAseq data revealed novel participating genes that were missed by BLAST analysis, facilitated aromatic metabolism pathway elucidation, and improved the existing genome annotation significantly. These pathways are being confirmed using metabolomics data. Finally, we will discuss the development of genetic tools to rapidly engineer this non-model system for the production of novel fatty acids such as ricinoleic acid and omega-3 fatty acids.

O7.2

Microwave pretreatment of corn and miscanthus stalks for integral chemical valorization

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Microwave (MW) technology is an innovative pretreatment to render possible integral biomass valorization in bioenergy and green chemistry applications. In most of the few reported studies, only bioethanol production from carbohydrates is investigated. However, high added value chemicals, specifically Ferulic Acid (FA), a vanillin precursor, can be recovered in a biorefinery context.
This work aims to study the effects of MW pretreatment on corn and miscanthus stalks, two underexploited agri-food by-product and dedicated crop feedstock respectively. In order to optimize FA recovery and to improve biomass biodegradability, we work with various mixtures of lignocellulosic substrates and solvents (water, ethanol, alkaline, acid) under different MW conditions (power density and temperature within different times).
MW effects are evaluated by a complete stalks characterization before and after treatment. Moreover FA and others carbohydrates liberated by enzymatic hydrolysis are quantified by HPLC. Results showed that FA release depends mainly on the solvent employed.

O7.3

Modified Hydrotropic Pretreatment of Eucalyptus at Alkali and Acidic Conditions for Enhancing Enzymatic Hydrolysis

Hongyan Mou

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Hydrotropic pretreatment is an environmental-friendly technology which can be applied for lignin removal from the lignocellulosic biomass for enhancing enzymatic hydrolysis. However, the pretreatment mechanism is still not completely understood. In this study, conventional hydrotropic pretreatment was modified with addition alkali or acid respectively to facilitated the lignin removal efficiency. The precipitated lignin recovered from the spent solution was analyzed by HSQC-NMR. The linkage of β-O-4 in modified hydrotropic lignin were retained better (>50%). Alkali-modified hydrotropic lignin contains 74.9% of linkage β-O-4. Compared with conventional hydrotropic method, surface lignin was reduced dramatically by modified hydrotropic method. In addition, the changes in the lignocellulose structure after modified pretreatments were more easily to be hydrolyzed, because the enzyme adsorption capacity improved significantly by modified hydrotropic pretreatment. As a result, the maximum glucose yield was obtained from acid-modified hydrotropic pretreatment.

O7.4

Engineering Bacterial Biocatalysts for Bioconversion of Lignin-Derived Compounds Into Bioplastic Precursors

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Lignin is a major renewable source of aromatics. Although there are efforts to valorize lignin using chemical catalysis, bioconversion by microorganisms offers the possibility to develop cost-effective, environmentally-friendly and regioselective processes, what has attracted a great industrial interest.
Pseudomonas putida KT2440 is a very well-known and genetically-amenable biocatalyst mainly recognized by its ability to degrade aromatic compounds. The KT2440 strain behaves as an ideal host for expanding the range of substrates that it can biotransform into added-value products through the recruitment of heterologous genes and system metabolic engineering strategies.
We present here the rational engineering of the ortho-cleavage beta-ketoadipate degradation pathway of P. putida KT2440 to develop a cell factory for the efficient channeling of lignin-derived compounds into PDCAs (pyridine dicarboxylic acids). This strategy could pave the way for the sustainable industrial production of polyesters bioplastics, replacing the current petroleum-derived phthalic acids by bio-phthalic acids derived from biomass.

O7.5

Wood-decay Polyporales fungi in lignocellulose bioconversion: genomics, interactions and decomposition mechanisms studied for sustainable bioeconomy

Taina Lundell[1], Mari Mäkinen[1], Hans Mattila[1], Tuulia Mali[1], Jaana Kuuskeri[1], Pia Laine[2], Olli-Pekka Smolander[2], Lars Paulin[2], Markku Varjosalo[2], Petri Auvinen[2]

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Enzyme activities in wood-supplemented cultures demonstrated the proficiency of Polyporales phlebioid clade fungi, which were further investigated for bioethanol production from recyclable waste lignocelluloses. Genome sequencing of Phlebia radiata aided in description of functional transcriptome and proteome of the white-rot fungus on spruce wood, and more recently, transcriptome under fermentative, ethanol producing conditions. Differentially expressed key genes were identified to elucidate the principal metabolic pathways and putative transcription factors operating under fermentative versus aerobic, respirative conditions. Our second concern is to elucidate brown-rot decay of wood conducted by the Polyporales species Fomitopsis pinicola and its interactions with white-rot fungi. In nature, fungal communities are under dynamic changes, and co-habitation of several species is common upon wood decay. Hyphal contacts and interactomes in decomposition of lignocelluloses and plant biomasses are challenging to investigate, but may offer us new tools for bioproduction and bioconversions promoting sustainable bioeconomy.

P01

Two Dichomitus squalens CE15 glucuronoyl esterase isoenzymes showing different characteristics

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Glucuronoyl esterases (GEs) catalyze the hydrolysis of the link between 4-O-methyl-D-glucuronic acid side residues of glucuronoxylan and lignin alcohols from lignin-carbohydrate complexes, and can therefore play a significant role as auxiliary enzymes in plant biomass saccharification for the production of biofuels and biochemicals. GEs belong to a carbohydrate esterase family 15 (CE15) in the CAZy database and only few fungal GEs have been characterized.
Using BLAST analysis more than 150 putative fungal GEs were identified, two of which were from Dichomitus squalens (DsGE1 and DsGE2). D. squalens is a white rot Basidiomycete, which is a promising source of new lignocellulolytic enzymes. Sharing only 30% amino acid sequence identity, DsGE1 and DsGE2 belong to two different sub-groups of CE15. Both genes were expressed in Pichia pastoris and the biochemical and saccharification properties of the recombinant proteins will be presented.

P02

Production, partial purification, immobilization and characterization of a pectinase produced by Aspergillus terreus

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Currently, the main challenges of biotechnology industries that use enzymes are to increase processes productivity and develop techniques that optimize the enzymatic catalysis. These requirements are essential to provide large-scale production and cost-effective formulation. In this context, enzymatic immobilization appears to be a possible alternative, as long as it improves the enzymatic stability and allows enzymes reutilization. The present work involved the production, partial purification, immobilization and characterization of the pectinase produced by Aspergillus terreus when grown in sugar cane bagasse. The immobilized enzyme showed higher activity in acidic pH when compared to free enzyme. The thermostability at 50ºC for free enzyme was only 5 minutes, while for the immobilized enzyme it was 30 minutes. The data presented are significant for the possible application of immobilized enzymes in biocatalysis processes that require high reaction temperatures over a prolonged period of time, and in more acidic environments.

P03

Development of a new laccase activity assay method using LC-MS based on 'lignin-like' compounds

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Laccase (benzenediol: oxygen oxidoreductases; EC 1.10.3.2; AA1) activity has traditionally been determined spectrophotometrically by measuring the oxidation of chemical compounds such as syringaldazine and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), also known as ABTS. The poor relation between these compounds and lignin provide little information about the true laccase oxidative mechanism. We have developed a highly sensitive, accurate methodology based on UPLC-MS to assess laccase activity on more "lignin-like" compounds also providing information about products formation and time-dependent reaction development. We tested the ability of different laccases to oxidize three different hydroxycinnamic acids (sinapic acid, ferulic acid and p-coumaric acid) and a dimeric lignin model compound, determination of kinetic parameters and products profiles evaluation. The results show that laccases exhibit highest activity on substrates containing methoxylated phenolic moieties, and that the product profile during the initial part of the reaction consists of a limited number of dimeric species derived from the substrate.

P04

Optimization of enzyme cocktails and process conditions for efficient saccharification of Norway spruce

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Efficient and sustainable use of biomass as renewable feedstock for heat, power and transportation is key to achieving the 2C climate goal proposed by UNFCCC1. These issues are addressed in the environment-friendly energy center Bio4Fuels with production of biofuels and value-added chemicals by catalytic and biochemical conversion of biomass as main targets.

Enzymatic hydrolysis of lignocellulosic biomass is a limiting step for making bioconversion processes economically feasible. Therefore, the Bio4Fuels project is focused on process development to improve saccharification of softwood at high dry-matter, assessing reactor technologies and reaction conditions.

Specific enzyme activities critical for the conversion of lignocellulosic biomass are being investigated, and this includes testing of hemicellulasses, lytic polysaccharide monooxygenases (LPMOs) and yet-to-be discovered novel enzymes. The recently elucidated LPMO mode of action demonstrating their reliance on H2O2 will be used to further optimize the saccharification conditions for optimal LPMO activity using various feeding strategies of the co-substrate.

P05

Characterization of rare-cutting xylanases for extraction of hemicellulosic polysaccharides

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Traditionally xylanases have been used for conversion of plant biomass into biofuel or other organic compounds. Therefore, the plant cell wall hemicelluloses are generally digested completely to the monomers. Recently also the use of xylooligosaccharides for food, feed and material science applications gained some interest. For extraction of these oligomers “rare-cutting” xylanases from the GH families 5, 30 and 98 can be applied. We studied a unique set of such xylanases from different bacterial and metagenomic origins, which have not been characterized yet. All enzymes were biochemically characterized and their hydrolytic products from certain industrial waste biomasses were identified using size exclusion chromatography and MALDI-MS. Preliminary data show that most of the studied enzymes indeed produce the desired xylooligosaccharides. Future efforts will validate their application in an industrial context.

P06

Biochemical and structural investigation of the CE15 family: Glucuronoyl esterases acting on recalcitrant biomass

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Glucuronoyl esterases (GEs) are a relatively new class of enzymes which cleave ester linkages between lignin and glucuronoxylan. GEs have been identified in many biomass-degrading microbes and are now classified into the Carbohydrate Esterase Family 15 (CE15). The CE15 family is diverse (as low as 20% sequence identity), however to-date only a few GEs from a small clade of the CE15 phylogenetic tree have been biochemically characterized and only two protein structures have been solved. To investigate the diversity of CE15 members, we have studied a broad range of proteins from across the phylogenetic tree. Enzymes were biochemically characterized and three-dimensional structures for two of the enzymes were solved. Analysis of the structures suggest possible binding sites for lignin fragments and xylooligosaccharides that have not been previously reported. Investigations into the molecular determinants supporting the potential binding sites is being pursued.

P07

Characterization and applications of AA3_2 family carbohydrate oxidoreductases in activation of hemicellulosic oligosaccharides

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The carbohydrate active enzymes from the auxiliary activities (AA) family 3 are glucose-methanol-choline oxidoreductases containing a flavin-adenine-dinucleotide-binding domain. Family AA3 has been divided into four subfamilies. Subfamily 2 (AA3_2) comprises both oxidases and dehydrogenases acting on carbohydrates or alcohols. AA3_2 carbohydrate-acting enzymes, glucose 1-oxidases, glucose dehydrogenases and pyranose dehydrogenases, have different substrate specificities and variable regioselectivities towards the monosaccharides and oligosaccharides.[1]
Currently hemicellulosic oligosaccharides are underused biomass fraction in the pulp and biofuel industry. These oligosaccharides could be used as starting material in biobased coatings, films, surfactants, and cross-linkers in composite materials. Utilization of these oligosaccharides via enzymatic pathway requires activation on the termini of the oligomers, leading to building blocks for re-assembly of a new material. In this work, we present the screening of oxidoreductases targeting the non-reducing end of hemicellulolytic oligosaccharides and the characterization of the oxidized carbohydrates.

P08

Biochemical characterization of laccases from the white-rot fungus Obba rivulosa

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Obba rivulosa (syn. Physisporinus rivulosus) is a selectively lignin-degrading white-rot fungus, which efficiently decomposes softwood. O. rivulosa genome encodes a full set of lignocellulose-degrading genes, making it an interesting candidate for plant biomass conversion. We have heterologously expressed two low pH laccases of O. rivulosa and their site-directed mutants in the methylotrophic yeast Pichia pastoris. The substrate specificities of these laccases as well as their thermal and solvent tolerance were determined. The oxidative efficiency of the recombinant O. rivulosa laccases towards non-phenolic lignin model compounds in presence of different types of laccase mediators will be discussed.

P09

Exploring laccase mediator systems with acidic recombinant enzymes from Obba rivulosa

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The lignin-degrading white rot fungus Obba rivulosa produces several laccases with technically interesting properties such as thermostability, thermal activation and extremely low pH optima. Heterologous expression of the O. rivulosa laccases in Pichia pastoris enables the detailed study of their properties and the evaluation of their potential as oxidative biocatalysts for conversion of wood lignin, lignin-like compounds, and soil-polluting xenobiotics. The enzymatic properties of the recombinant laccases demonstrated low pH optima (pH 3-3.5). We tested the efficiency of these recombinant enzymes in various laccase mediator systems (LMS). The results showed that LMS can be used to selectively oxidize different types of non-phenolic substrates. We further compared these results with the performance of LMS-oxidation on biorefinery-sourced poplar lignin.

P10

Visualising the effects of non-catalytic fungal proteins on enzyme accessibility using a 3D lignocellulose model.

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In an effort to uncover new proteins that can be used to modify plant polymers for the synthesis of high-value bio-based materials, we developed a novel 3-dimensional lignocellulose assay for enzyme accessibility. After proof of concept, this model was applied in the characterisation of non-catalytic fungal proteins. Comparative transcriptomics of the white-rot fungus Phanerochaete carnosa led to the identification of protein sequences homologous to loosenin and cerato-platanin. Similar to expansins, both protein classes have been noted for their cell wall loosening ability, making them interesting targets for the chemical and biophysical modification of plant polysaccharides. Cerato-platanin- and loosenin-like proteins were recombinantly expressed in Pichia pastoris and functionally characterised. By tracking the action and migration of a 'reporter' enzyme through a defined polysaccharide-matrix, effects of these non-catalytic proteins on lignocellulose accessibility and enzyme activity were investigated.

P11

Heterologous expression of Fomitopsis pinicola lignocellulolytic enzymes in Trichoderma reesei

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Basidiomycete brown-rot fungi play an important role in the carbon-cycle of forest ecosystems as extremely efficient degraders of biomass. To this purpose brown-rots have evolved a fast and more cellulose specific set of enzymes compared to their white-rot ancestors. In addition to a rather small set of hydrolytic carbohydrate-active enzymes, this group of fungi utilize sophisticated and partially unresolved mechanisms to generate reactive low-molecular-weight oxidants for the efficient degradation of lignocellulose.
This work focusses on the oxidoreductase machinery found in Fomitopsis pinicola, a saprotrophic wood decay fungus of the order of Polyporales. Lignocellulolytic enzymes e.g. LPMOs, laccases, peroxidases, GHs, etc. were identified using a genomic approach and verified by secretome studies. To investigate the interactions between different enzymes and to elucidate the reaction mechanisms involved, selected enzymes were heterologously produced for further characterizations using Trichoderma reesei as expression system.

P12

Recombinant expression and characterization of lignocellulose-active enzymes from Phanerochaete chrysosporium

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The white-rot basidiomycete Phanerochaete chrysosporium secretes a diverse arsenal of biocatalysts capable of lignocellulose break-down that act in a concerted manner. In this study, we investigate the distribution and interaction of an assorted set of oxidoreductases on synthetic and natural substrates. This requires the recombinant production of cellobiose dehydrogenase, lytic polysaccharide monooxygenase, lignin peroxidase, manganese peroxidase, etc. As expression hosts we chose the yeast Pichia pastoris for steady-state kinetic measurements and the more complex fungus Trichoderma reesei to produce enzymes with a glycosylation pattern similar to the native one for further characterization. Challenges include the creation of stable transformants as well as screening for expression. The produced enzymes were modified with fluorescent tags and their localization and also interaction on lignocellulosic samples was investigated by fluorescence microscopy.

P14

Quantitative kinetics of radicals in lignin – a real time laccase assay

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Reflecting laccase activity on lignin is analytically time consuming. Currently laccase activities are measured by reaction with soluble low molecular weight molecules (e.g. ABTS and syringaldazine), which poorly resemble the lignin polymer. Electron paramagnetic resonance spectroscopy (EPR) was employed for real time measurement of laccase catalyzed radical formation in lignin by two different fungal laccases, derived from Trametes versicolor and Myceliophthora thermophila, respectively. Experimental data verified that direct quantitative kinetics of laccase action on solid lignin of different origin can be provided by this method. The method was employed to assess the effect of the redox potential of the T1 cupper site in laccase on the radical formation in lignin. Additionally, the effect of selected mediators on the radical formation in the lignins facilitated by the laccases was measured. EPR methodology present a new type of enzyme assay that provides insight into the interaction between laccases, lignins and mediators.

P16

Optimizing process conditions for industrial lignocellulose degradation using a fungal enzymatic cocktail

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Enzymes secreted by fungi can be harvested and engineered for industrial conversion of lignocellulosic substrates and thus contribute to solving one of the major challenges in biorefining. We have assessed the lignocellulose-degrading potential of an enzymatic cocktail developed from the secretome of Thermoascus aurantiacus [1]. Next to modifying parameters such as reaction temperature and percentage dry matter of substrate, we put particular emphasis on parameters that potentially affect the function of lytic polysaccharide monoxygenases (LMPOs) [2], such as the availability of H_2O_2 [3] and reducing agent. Thus, we are trying to achieve optimal processing conditions for the industrial degradation of sulfite-pulped Norway spruce, with the additional aim of understanding how to maximize the potential of a Thermoascus aurantiacus secretome enzyme cocktail.

P17

Effect of thermochemical pretreatment severity on lignin inhibition in the enzymatic hydrolysis of lignocellulosics

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Lignocellulosics is an abundant raw material and an alternative mean for production of energy carriers and chemicals. Thermochemical pretreatment is commonly used for lignocellulosic biomasses to open up the cell wall structure and to enable enzyme access to the structural polysaccharides. Lignin restricts the enzymatic hydrolysis by various mechanisms. Non-productive adsorption onto lignin is the major inhibitory mechanism. This study aims to elucidate how the pretreatment severity influences the inhibition by lignin during enzymatic hydrolysis. Spruce and wheat straw were hydrothermally pretreated at 180, 200 and 220 °C with or without addition of an acid catalyst. Unpretreated and pretreated lignins were isolated after ball milling and characterised chemically. This paper discusses the effect of different pretreatment conditions on the inhibitory nature of lignin during enzymatic hydrolysis and the adsorption of enzymes on the lignin fraction.

P18

Structural characterization of Glucoronoyl Esterases from Opitutus terrae

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Improvements in biomass conversion can advance renewable resources technologies. Cellulose and hemicelluloses can be used in biofuel production, but as they are trapped in a complex matrix, they require concerted action of many enzymes and/or physico-chemical treatment for full breakdown into component sugars.

Glucuronoyl esterases (GEs), microbial enzymes classified into the Carbohydrate Esterase family 15 (CE15), break an ester bond between 4-O-Me-D-glucuronic acids of glucuronoxylan and alcohols of lignin and may aid in biomass exploitation. To-date few GEs have been biochemically characterized and only 3 GE structures (2 of fungal origin) have been determined. Opitutus terrae, a soil bacterium, has 4 different CE15 genes within its genome coding for OtCE15A through D, here biochemically characterized as GEs.

The structure of OtCE15A has been solved by crystallography, showing a typical α/β hydrolase fold with conserved catalytic triad. Binding interactions with aromatic lignin substituents could be facilitated by a conserved phenylalanine residue.

P20

The hydrolytic activity of α-expansin from peach

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Almost all of plants and a part of bacteria have small protein, called Expansins, which are considered to work at the reconstruction of plant cell wall. Expansins have similarity to endo-glucanases belonging to Glycoside Hydrolase (GH) family 45, wherareas this protein family has been believed without hydrolytic activity. Here we studied the hydrolytic activity of peach's expansin (PpEXP1), which contribute to the ripening of peach fruit.
PpEXP1 was heterologously expressed in the yeast Pichia pastoris, and tested for the reactivity against cellooligosaccharides, carboxymethyl-cellulose (CMC), xyloglucan, phosphoric acid swollen cellulose (PASC), and cellulose extracted from peach cell wall. PpEXP1 did not reacted against cellotriose and cellotetraose, but it hydrolyzed cellopentaose and cellohexaose. Moreover, this protein showed hydrolytic activity not only against CMC, xyloglucan, PASC, but also against cellulose isolated from peach. Based on these results, we conclude that PpEXP1 is an endoglucanase.

P21

Function of an α-L-arabinofuranosidase in the cellulosic biomass degradation by the white-rot basidiomycete Phanerochaete chrysosporium

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For efficient enzymatic saccharification of polysaccharides, enzymes degrading side chains are important in addition to the degradation of main chain. α-L-Arabinofuranosidase is an enzyme that cleaves α-L-arabinofuranoside residues in arabinoxylan, pectic arabinan and arabinogalactan, and the white-rot basidiomycete Phanerochaete chrysosporium produces the enzyme in cellulolytic culture, whereas the content of arabinose is quite small in hard wood, natural substrate of this fungus. In the present work, we cloned the gene encoding a glycoside hydrolase family 51 α-L-arabinofuranosidase from the fungus, designated PcAraf51A, and heterologously expressed and characterized to understand the function of this enzyme. Among arabinose-containing polysaccharides, PcAraf51A was highly active on arabinoxylan from monocots, and the gene was also highly expressed when the arabinoxylan was used as a sole carbon source. We will discuss about its function of the enzyme with the comparison of its gene distribution in the genome of filamentous fungi.

P22

Genetics of induction of pentose catabolism in Aspergillus niger

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The filamentous ascomycete Aspergillus niger degrades plant cell wall polysaccharides to obtain monomeric sugars that can serve as a carbon source. In nature, D-xylose and L-arabinose are the most abundant monosaccharides after D-glucose, being the major components of xylan, pectin and xyloglucan. Therefore, the pentose catabolic pathway (PCP), related to these carbon sources, is highly important for A. niger. Most of the genes involved in the PCP have already been characterized, but only assumptions regarding the induction of the system have been reported. Thus, the aim of this study was to obtain a better understanding of the pentose catabolism, and the induction mechanism of the PCP genes and the arabinanolytic and xylanolytic regulatory systems of A. niger. To this end, phenotypic and transcriptomic analysis, intracellular accumulation of metabolites and enzymatic activities of single and double gene deletion mutants of PCP genes were conducted. Highlights of this study will be presented.

P23

Genetic engineering of the Aspergillus niger regulatory system using the CRISPR/Cas9 technology

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Although CRISPR/Cas9 technology has already been applied in the industrial workhorse Aspergillus niger, little is known about the efficiency of various types of genome editing using this technology. We used the CRISPR/Cas9 system in the Aspergillus niger N593 pyrG- Δku70 strain, in which the double strand breaks were repaired through homology-directed recombination. First, we knocked-out the genes encoding the xylanolytic regulator, XlnR and arabinolytic regulator, AraR both individually and in combination. The mutant strains' had a reduced ability to grow on xylan and arabinan, respectively, confirming the successful deletions. Additionally, we made a single nucleotide mutation in the terminal region of the xlnR gene, which resulted in a mutant with a constitutively active XlnR regulator. This genome editing system has provided us with the means to study the role of these transcriptional regulators on a new level.

P24

Dichomitus squalens as a model white-rot basidiomycete for plant biomass degradation

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The white-rot fungus Dichomitus squalens is an efficient soft- and hardwood degrader with a tailored enzymatic response to various plant biomass types matching their cell wall composition. This feature together with its recently established genetic transformation system and genome sequences for four strains makes D. squalens an ideal basidiomycete to study modification and degradation of plant biomass. We have previously described differences between the ability of mono- and dikaryotic strains of D. squalens to grow on and degrade plant biomass. To study this in more detail, we grew three monokaryotic and four dikaryotic D. squalens strains on spruce wood and analysed the cultures for their transcriptome, proteome and metabolome, demonstrating differences between the strain lineages as well as between mono- and dikaryons. Highlights from this study will be presented.

P25

Comparative genomics of white-rot phlebioid fungal species and transcriptomics of the Polyporales fungus Phlebia radiata

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White-rot Basidiomycota fungus Phlebia radiata is an efficient degrader of plant cell wall and presents high applicability in biotechnological processes such as production of bioethanol from lignocellulose waste materials in a single-step process. After genome sequencing, comparative genomics study of P. radiata together with six other phlebioid species was conducted with special emphasis on the CAZy gene content of the genomes. This study revealed shared and specific properties of closely related fungi with seemingly similar lifestyles. Several holistic transcriptomics studies of P. radiata grown on different substrates (solid spruce wood, liquid malt extract medium, solid lignocellulose waste materials) and atmospheric (aerobic or fermentative) conditions was conducted by RNA-seq. Transcriptomics data enabled clustering of P. radiata genes according to expression profiles, identification of co-regulated genomic clusters of genes and investigating the regulatory mechanisms of especially CAZy genes.

P26

An Aspergillus niger colony locally adapts its molecular responses to spatially separated substrates

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Saprotrophic fungi, such as Aspergillus niger, grow as mycelial colonies that are often considered uniform entities. To test this uniformity, we analysed pie-slice sections of a colony grown on spatially separated substrates (glucose, wheat bran, sugar beet pulp) using transcriptomics, proteomics and metabolomics. The colony tuned its response to the local carbon source composition. Plant biomass degrading CAZymes and intracellular carbon catabolic enzymes were more abundant in parts of the colony containing the corresponding sugars; e.g. a stronger pectinolytic response was observed in the part of the colony grown on the pectin-rich sugar beet pulp. Our results argue against a situation in which small molecules are transported efficiently through the colony and favour high diversity within the fungal colony in natural biotopes, where the substrate is typically heterogeneous. It also demonstrates the high level of plasticity of A. niger in response to the composition of the prevailing lignocellulose.

P27

Enzyme activities and transcription in Pleurotus ostreatus ligninolytic mutants on rice straw medium

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Pleurotus ostreatus is one of white-rot fungi frequently used for molecular genetics studies of lignocellulose degradation. Recently we identified some genes, pex1, wtr1, wtr2, hirA and chd1, in which mutations cause defects in the ligninolytic activity when they are grown on beech sawdust medium. However, it remains unclear whether these mutations also cause defects in the ligninolytic activity when grown on other lignocellulosic substrates. In this study, we examined the effects of these mutations on the extracellular enzyme activities and transcriptional expression of mnp and vp genes on rice straw medium. It was revealed that Mn2+-dependent peroxidase activity and vp/mnp transcriptions were inactivated overall in these mutants, despite the pattern of transcriptional expressions of vp/mnp on rice straw medium is significantly different from that on sawdust medium in wild-type strain. Based on this finding, we are now conducting comparative transcriptome analyses.

P28

How biomass pretreatment modifies functional diversity of a lignocellulolytic microbial consortium? A metaproteomic assessment.

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In order to optimize the carboxylate production from lignocellulosic biomass, substrate pretreatment is frequently used to improve bioconversion rates. While the impact of pretreatment on substrate features (structure, composition) and enzymatic hydrolysis has been widely studied, its impact on the functional diversity of microbial communities has been poorly described. Here, the dynamic of the functional diversity (Carbohydrate-Active enZymes - CAZymes) of a lignocellulolytic microbial consortium was studied by metaproteomics. Raw and dry-chemically pretreated wheat straw were used as substrate for carboxylate production. Proteins-based taxonomy was similar between raw and pretreated substrates, dominated by Bacteroidetes and Firmicutes members. A faster carboxylate production rate and an increase of xylanase activity for chemo-mechanical pretreatments were correlated to the abundance increase of Bacteroidetes related proteins belonging to GH43 family at the early steps of bioconversion. Moreover, metaproteomics suggest a functional interplay between Bacteroidetes and Firmicutes phyla, which produced enzymes belonging to different CAZymes families.

P29

Interactions of wood-decay fungi: enzyme activities, gene-expression and spruce wood decomposition

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Fungal communities in decaying wood are dynamic. The influence of Fomitopsis pinicola, a common brown-rot Polyporales species encountered in boreal forests, on the white-rot species Phlebia radiata and Trichaptum abietinum, was studied in co-cultures on spruce wood. Activities of lignocellulose-decomposing enzymes were followed together with gene expression studies. Fungal growth and colonization was followed by ergosterol analysis and electron microscopy. In the presence of F. pinicola, wood carbohydrate consumption and significant wood-mass loss occurred within three months in the co-cultures. Sugars from the wood substrate were accumulating with cellulolytic activities, oxalic acid, released volatile organic compounds and promotion of Fenton reaction, pointing to aggressive and mainly non-enzymatic decomposition of wood polysaccharides by F. pinicola. T. abietinum and P. radiata secreted an array of enzyme activities including lignin-modifying oxidoreductases. Our results point to the significance of fungal species-species interactions for wood-decomposition processes and carbon cycling in the forest ecosystems.

P30

Proteomic Insight into the Cellulose Degradation Systems of Cytophaga hutchinsonii and Sporocytophaga myxococcoides

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Cytophaga hutchinsonii and Sporocytophaga myxococcoides are both Gram-negative, aerobic, mesophilic, cellulose degrading bacteria belonging to the phylum Bacteroidetes. Despite both of these organisms having been isolated almost a century ago, they remain poorly studied with the proteins responsible for cellulose degradation largely unknown. Both organisms utilize cell associated enzyme machineries to degrade crystalline cellulose but do not contain any expected scaffoldin or dockerin proteins as in the well-known complexed cellulosome system. Therefore, we have employed proteomic analysis in order to identify which proteins are present and abundant during growth on crystalline cellulose, and to assess their putative roles in cellulose hydrolysis. Further, we have been able to infer the localization of these proteins based on their abundance in specific cellular fractions. Taken together, the abundance and localization of proteins putatively involved in cellulose degradation gives important insights into the cellulose degradation mechanisms in both C. hutchinsonii and S. myxococcoides.

P31

Lignin degrading enzymes from the ascomycete fungus Phoma herbarum

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While extensive research has been conducted on the lignin degrading mechanisms utilized by basidiomycete white-rot fungi, much less attention has been given to ascomycete fungi in this regard. However, it has been demonstrated that there are also ascomycete species capable of depolymerizing lignin to some extent. One ascomycete species studied regarding lignin degradation is Phoma herbarum, a strain of which was previously shown to depolymerize lignin. Through whole genome sequencing and gene annotation we have gained some insight into the mechanisms this fungus potentially uses for lignin degradation, this serving as a starting point for further functional studies of the enzyme activities produced by this fungus. Since many enzyme production and consolidated bioprocessing platforms are based on ascomycete strains, sourcing relevant enzymes from ascomycete species can provide useful targets for biotechnical applications while potentially bypassing some of the problems involved in heterologous expression of basidiomycete enzymes.

P32

Strain improvement for lignocellulolytic enzyme production in Trichoderma reesei

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Lignocellulosic biomass is the most abundant renewable resource and has enormous potential for sustainable production of biofuels, biomolecules and biomaterials. Several enzymatic activities are required to hydrolyse plant biomass into fermentable sugars. The efficiency of the cellulolytic enzyme system in the hydrolysis of plant material depends not only on properties of individual enzymes but also on the composition of the enzyme cocktail. This work describes strategies to improve T. reesei as the production host for plant biomass hydrolysis.
We optimized T. reesei strains by expression of heterologous β-glucosidase and overexpressing lytic polysaccharide monooxygenase (LPMO). The strains produce a very potent lignocellulose hydrolysis enzyme cocktail comparable to commercially available mixtures. The strains were improved for total protein production by overexpression of the regulatory gene xyr1, allowing more than 100 g/l total protein to be secreted. We introduced modified XYR1 regulatory factor and generated a strain that produces up to 50 g/l enzyme mixture on glucose media.

P33

Search for production of high-quality lignin from technical lignins by enzymatic catalysis

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It is challenging to develop commercially viable methods for turning lignins into valuable products in biorefineries. A prominent method for biorefining is to apply biotechnological approaches. In the case of biotransformation, the limiting factors are low solubility and slow transformation rates. Basidiomyceteous white rot fungi are naturally occurring lignin degraders and therefore we have studied the ability of O. rivulosa and P. radiata fungal species to incubate with technical lignins. We examined how the pH of culturing conditions and the presence or absence of different nutrients affect the expression of enzymes and modification of the lignin's chemical structure. The changes in the chemical structures were analyzed by SEC, Py–GC–MS, NMR, and elemental analysis. The results can be utilized for the development of more efficient, controlled and faster methods to modify low value technical lignins into high value bio-based products.

P35

Changes in Eucalyptus cellulose structure after different alkalization conditions

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Holocellulose samples isolated from Eucalyptus benthamii, E. nitens and E. smithii were treated with NaOH at different concentrations (up to 35%) and temperatures (25ºC and 80ºC), with the aim to determine their structural variations. X-ray diffraction analysis was used to determine crystallinity index (CrI), cellulose II (Cell-II) conversion, and crystallite size. Results showed an increase of CrI after 0.5% NaOH treatments and it was observed that Cell-II conversion started after 10% NaOH treatments at both temperatures, being lower the Cell-II content in E. benthamii and higher in E. nitens. Depending on the NaOH concentration, crystallite size varied, being observed an increase for cellulose I (Cell-I) crystal size from 3.5 to 5.0 nm, while Cell-II crystals were more homogeneous (4.7-5.1 nm). Significant cellulose structural differences were observed, which depended of the reaction conditions and cellulose source. This may be relevant in the processing and quality of cellulose derivatives.

P36

The contribution of CWPO-C to primary stage of plant growth and organogenesis

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Cationic cell-wall-bound peroxidase (CWPO-C) from poplar has been believed as a lignification-specific peroxidase, but direct evidence demonstrates the role of CWPO-C remains unachieved. To promote better understanding about CWPO-C functions, transcriptional analysis of CWPO-C using laser microdissection, gene expression quantification and reporter gene techniques were performed. The results showed that CWPO-C was expressed in most of young tissues including xylem of upper stem, but scarcely expressed in interfascicular fibers and undifferentiated tissues such as cambium. Heterologous overexpression of CWPO-C in Arabidopsis inhibited plant growth and caused stem curvature. In addition, CWPO-C was expressed in the outer side of the curved stem that was subjected to gravity stress. These results indicate that CWPO-C plays a role in cell elongation and differentiation; suggesting a new aspect to the role of CWPO-C. CWPO-C may contribute to catabolism of plant hormones such as auxin, involved in cell elongation, differentiation and geotropism.

P37

Engineering laccase activity for polymerization of lignins as renewable binders in paper coatings

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To replace fossil-based styrene-butadiene (SB) latex as binders in conventional paper coating formulations, we developed a novel lignosulfonate (LS) pre-treatment and polymerization process. Laccases from Trametes hirsuta and engineered laccase from Aspergillus flavus were used for enzymatic polymerization of LS. We demonstrated that continuous supply of oxygen is sufficient for polymerization of LS, thus making mediators redundant. Both enzymes were able to extensively polymerize lignosulfonates resulting in a 13-fold increase in MW up to 170 kDa. The application as binders in paper coating formulations resulted in improved printing properties (reduced picking) by fractionating LS before polymerization. Water retention properties of the paper coatings were comparable to those obtained with reference latex. Cross sectional fluorescence microscopy images showed that ultrafiltration prior to laccase polymerization reduced penetration of the polymerized lignosulfonates into the base paper to 33 % and additionally reduced polymerization time from 6 to 2 h.

P38

Unique aspects of TEMPO-oxidized cellulose nanofibril/mixed-linkage β-glucan bionanocomposite gels

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Bio-based gels are widely used in food, pharma, and medical applications as rheology modifiers, drug carriers, and filler materials due to biodegradability and non-toxicity. Cellulose, can be processed to nano-fibrils and -crystals with unique mechanical properties, holds significant promise for these applications. In this work, we present the preparation, rheological characterization, and nanoscale morphology of ultralow solid content TEMPO-CNF gels by introduction of a soluble plant polysaccharide, MLG as a physical cross-linking agent. Rheology of these gels revealed that the cg of MLG/TEMPO-CNF followed a power-law function. In light of these observations, it is proposed that non-covalent cellulose-MLG interactions, analogous to those occurring within plant cell walls, drive gel formation. Furthermore, gel formation is dependent on fulfilling steric requirements relating inter-fibril distance and number of MLG chains in the system. The ability to tune gel physical properties by controlling the amounts of CNF and MLG opens new avenues for applications.

P39

Improving retting management to expand the industrial use of plant fibers

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The use of plant fibers as hemp (Cannabis sativa L.) has spread to high-value applications as natural fiber use to replace synthetic fibers in composites materials. However fiber quality is still a critical issue for industrial uses and one major challenge concerns the retting process achieved in the field in Europe (dew-retting). This process is a selective biodegradation facilitating fibers extraction while preserving quality. Retting is highly weather-determined and relies on empirical knowledge.
To deeper understand this natural and weather-dependant process, we have designed a new experimental set-up to perform dew-retting under controlled environmental conditions. This system enabled to carry-out hemp dew-retting with different climatic scenarii and biomass quality. The experimental design allows to quantify the induced effects of changes in environmental and biomass quality on the duration and the dynamics of retting. These data will allow improvement of retting management options for high quality fibers.

P40

Unravelling the Aspergillus niger aromatic metabolic pathways

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Aromatic compounds derived from lignin are of great interest for biorefineries. These compounds have applications in many industries, such as biochemical building blocks for bioplastics or (bio) chemicals, or as antioxidants, flavor agents or preservatives in the food industry. For most microorganisms, aromatic compounds have a toxic effect already at low concentrations, and hence these compounds are quickly degraded to non- or less toxic compounds. Many possible pathways have been described for fungi, but these observations are scattered over different species and only a few fungal aromatic metabolic enzymes are characterized. In order to explore aromatic metabolic pathways and to identify the novel fungal aromatic metabolic enzymes, we created an Aspergillus niger ferulic acid adaptive evolution mutant (FA6) by growing A. niger N402 on incrementally increasing concentrations of ferulic acid. Using this mutant, we were able to identify the genes of several aromatic pathways, some of which will be presented.

P42

Biomethane production from lignin-rich materials

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Lignin valorization has been considered the key to improve the economy of modern biorefineries focused on cellulose. Anaerobic digestion could be used for the conversion of lignin-rich materials to biomethane in order to valorize lignin-rich residues. However, lignin is very recalcitrant and its depolymerization under anaerobic conditions is very slow, which would need to be enhanced for efficient conversion of lignin into biogas. It has been shown that the direct addition of oxidative enzymes, such as lignin and manganese peroxidase, to anaerobic digesters could enhance the biogas production from wastes rich in lignin. Nevertheless, these enzymes require hydrogen peroxide as a co-substrate in order to break down lignin. In this work, we evaluate the anaerobic digestion and biomethane potential of different lignin-rich materials from birch and spruce in the presence of cow manure and the effect of hydrogen-peroxide and lignin-active enzymes addition.

P43

Biopigments production from Pichia kudriavzevii grown on sugarcane bagasse hydrolysate

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Biopigments are environmentally friendly molecules associated with several health benefits. Pichia kudriavzevii, a versatile yeast, was used in the present study for biopigments production, growing on sugarcane bagasse hydrolysate (SBH), obtained by enzymatic hydrolysis of bagasse pretreated by sodium hydroxide. P. kudriavzevii cells were cultured in semi-synthetic media (based on glucose and xylose in different concentrations) and in SBH. Samples were periodically taken to assess cell growth and sugars consumption. The experiments were conducted at 30°C, in Erlenmeyer flasks. In semi-synthetic media, maximum xylose and glucose consumption values were 64.35% and 91.68% respectively. When SBH was the carbon source, 100% of glucose and 77.77% of xylose consumption were observed, proving the hydrolysate as an amenable source of biopigments production. Thus, SBH has shown as a promising renewable carbon source for biopigments production by P. kudriavzevii, with potential for process development for biorefineries. Acknowledgements: FAPESP (process 2016/103636-8).

P44

Aureobasidium pullulans LB83 as a potential cellulase producer for applications in solid state fermentations

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The lignocellulosic by-products generated in the processing of agricultural products are rich in carbohydrates, such as cellulose and hemicellulose, and due of this can be used as raw materials for various biorefinary processes . Among the microorganisms cellulase producers Aureobasidium pullulans, a black yeast, has been highlighted as a possible cellulolytic enzymes producer, enabling the direct conversion of cellulose to bioproducts of economic interest. In present study, cellulase production by A. pullulans LB83 strain was evaluated in Petri dish tests with carboxymethylcellulose as carbon source. After 24 h of incubation at 30 °C, the growth of the yeast and a halo (2.05 cm in diameter) were observed and indicated the cellulase production by this strain. These results shows the potencial this yeast strain in the bioprocess for direct conversion of cellulose in bioproducts, for application mainly in solid state fermentations.

P45

Unaltered lignin production through the optimized organosolv fractionation of Miscanthus sacchariflorus with various precipitation methods

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Lignocellulosic biomass is the most abundant organic materials in nature, which accounting for about half of the global biomass, and an efficient fractionation of lignocellulosic biomass into its main constituents (cellulose, hemicellulose, lignin) is a prerequisite for used as a feedstock in a biorefinery. Organic solvent pretreatment is a promising method for the fractionation of lignocellulosic biomass because of its inherent advantages, like high purity lignin production and solvent recycling. In this study, the process variables of organosolv fractionation of lignocellulosic biomass including reaction temperature (170 ºC), reaction times (2.5 – 60 min), ethanol concentrations (50 - 70%(v/v)) and sulfuric acid concentrations (0.025 - 0.4%(w/v)) was optimized. Also, the recovery yield, purity and structural characteristics of lignin generated by various precipitation methods including dilution, evaporation and spray drying were evaluated.

P46

Optimization of organosolv process for the effective fractionation and utilization of rice husk

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Rice husk is one of the most generated agricultural byproducts in the world and is being studied as a source in the field of biorefinery. The purpose of this study is to improve the utilization of rice husk by separating the main components (glucan, xylan, lignin and silica) of rice husk minimizing the decomposed products. The ethanol organosolv process was carried out at various temperatures, times, ethanol concentrations and sulfuric acid concentrations. At the optimized condition (180 °C, 60 min, EtOH conc. of 60% (w/v), and sulfuric acid conc. of 0.2% (w/v)), extraction yields of xylan and lignin were > 70% and > 50%, respectively, and enzymatic digestibility of fractionated rice husk was > 80% (at 15 FPU/g-glucan enzyme loading). The silica content of remaining solid was about 50%, which was about 5 times higher than the raw sample (11%), and the purity of silica was > 99.0%.

P47

Cellulase production by Aureobasidium pullulans

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Production of efficient cellulase titers followed by their application in lignocellulose biomass hydrolysis have a crucial role in the overall success of biorefineries. In-house enzyme production saves the production cost of bioethanol and biochemical considerably. Considering this point, we have studied the cellulase production potential of one of the most versatile microorganism-Aureobasidium pullulans, which is considered as yeast like fungi microorganism. A. pullulans was grown in Mandels medium supplemented with 40 g/l of carboxymethyl cellulose (carbon source) for 96 h under submerged fermentation conditions (temperature 30 0C, 150 rpm, inoculum size 1.6 x108 CFU/ml). Maximum CMCase activity of 7.42 U/ml (µmol / min. mL substrate) was observed after 60 h of incubation. Preliminary results of this study show the cellulase production by A. pullulans under submerged fermentation conditions.

P48

Effect of different pretreatments on the composition and sugar recovery from spent coffee waste

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Spent coffee ground (SCG) is the by-product of coffee brewing. It is rich in oil and polysaccharides and can be a potential raw material for biobased chemicals. Pretreatments are prerequisite for efficient extraction of sugars from SCG. The present investigation draws light to the effect of various pretreatments such as AFEX, microwave, plasma, dilute acid, ferric chloride, potassium permanganate, microwave, organosolv, steam explosion and ultrasonication on the composition and subsequent sugar recovery from SCG. The pre-treated samples were subjected to enzymatic hydrolysis. The hydrolysates obtained were subjected to reducing sugar estimation along with analysis of any inhibitory compounds (furfural, hydroxymethyl furfural). Comparative analysis indicated that acid-acetone pretreatment proved to be the best pre-treatment method resulting in a reducing sugar content of 321.2 mg/g of SCG which 1.5-fold was higher than control (203.4 mg/g of SCG). It was concluded that acid-acetone pretreatment was the best strategy for sugar recovery from SCG.

P49

Optimisation of thermostable α-amylase production from B. stearothermophilus using Brewer's spent grain as carbon source

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α-amylases are enzymes that hydrolyse α-1, 4 glycosideic linkages in starch into glucose, maltose or oligomeric mixtures and has been widely used in industrial production. Brewer's spent grain (BSG) is an important by-product of the brewery industry. It is highly rich lipids, proteins and polysaccharides. The polysaccharide fraction of BSG can be processed to formulate media for bacterial enzyme production. In the present study, a thermophilic amylase was produced using pretreated BSG and hydrolysate employing Bacillus stearothermophilus as the fermentative microbe. A Taguchi L-9 array was designed to optimise the parameters for enzyme production. Three parameters with three levels viz. solid-to-liquid ratio, pH and time were chosen: it was found that a high S/L ratio (0.8g/10), alkaline pH (8) and a reaction time of 36h resulted in maximum enzyme activity (173.99 U/ml) which was 2.13 fold higher than the enzyme activity recorded using synthetic media (81.60 U/ml).

P50

Probiotic fermentation of brewer's spent grain for the potential application as animal feed

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Brewer's spent grain is an important by-product of the brewing industry. High in proteins, carbohydrates and fats, BSG finds wide applications in animal feed. Fermented products have unique functional properties imparting some health benefits to consumers. Microbial fermentation using probiotic may improve the nutritional value of BSG by altering the composition. In the present study, dried BSG along with hydrolysate was subjected to submerged fermentation process to produce lactic acid using Lactobacillus plantarum. Fermentation was conducted at 50°C maintaining a reaction volume of 50 ml. The process was optimised using a Box-Behnken design involving solid to liquid ratio, pH and time as parameters. The obtained optimum conditions for lactic acid production were 2.88g of BSG/L of reaction volume, a fermentation time of three days maintaining a pH of 7.0. Proximate analysis conducted before and after fermentation determined the potential of fermented BSG as animal feed.

P51

Sequential procedure for xylan extraction from sugarcane bagasse: impact on enzymatic xylan and cellulose hydrolysis

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Many processes have been suggested for xylan extraction from sugarcane bagasse. Uses of xylanases in alkaline sulfite preteated bagasse offers the possibility of extracting xylans relatively free of lignin. To extract xylan fractions with varied DP, a sequential process was exploited. The first fraction of xylan was obtained by cold alkaline extraction (10% m/m NaOH). These conditions allowed for complete deacetylation of the bagasse and 14% of xylan solubilization, which was precipitated with 30% (v/v) ethanol. To the supernatant, sodium sulphite (5% m/m) was added to pre-treat the residual sugarcane bagasse (2 h/120°C). The sequential process allowed the removal of 85% of lignin, producing a carbohydrate enriched solid, which was treated with commercial xilanase for 6 and 24 hours, releasing 25% and 43% of the residual xylan, respectively. The final solids presented low recalcitrance being suitable for complete enzymatic hydrolysis of the cellulose fraction.

P52

Separate hydrolysis and fermentation of rice straw with thermotolerant Kluyveromyces marxianus strain

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In the present work, the potential of rice straw (RS) for ethanol cellulosic production by separate hydrolysis and fermentation (SHF) was evaluated. RS was processed in three steps: 1) alkaline treatment, for acetyl removal (deacetylation); 2) acid treatment, for hemicellulose separation and 3) enzymatic hydrolysis to convert cellulose in glucose. The saccharification process, which was carried out in fed-batch mode (solid content of 24% w/v and enzyme load of 29.5 FPU/g of cellulose) resulted in high cellulose conversion (78%) being obtained a maximum glucose concentration of 109 ± 8 g/L, after 48 h. In the fermentation step, Kluyveromyces marxianus produced 32 g/L ethanol (efficiency of 67% and volumetric productivity of 3.1 g/L.h). Considering the ethanol production from various types of lignocellulosic biomass at high-solids content, these results are very attractive, especially the values of ethanol productivity. However, further studies are still necessary to improvement of enzymatic and fermentative process.

P53

Novel molecular tools for recovering valuable aromatic compounds from lignin streams

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Industrial lignin side-streams provide a potential source of multi-purpose aromatic compounds. Currently, these complex technical lignins remain underutilized, partially due to the lack of convenient methods and tools. In our study, a novel approach for the recovery of aromatic compounds was developed by utilizing single-chain variable fragment (scFv) antibodies. Three hydroxycinnamates (coumarate, ferulate and caffeate) were studied because of their abundance in lignin-based materials as well as their similar chemical structures. Specific and selective binders towards these molecules were isolated from a synthetic scFv-library by phage display technique and characterised. As a proof of concept study, a caffeate binder was employed to recover caffeic acid from spiked samples, including a simulated solution of the aromatic compounds, Kraft lignin and rice straw hydrolysate. As a result, high recovery and purity ratings for the targeted molecule were achieved indicating scFvs as a potential toolset for lignin valorisation.

P54

Lignin-derived molecules as substrate for novel cell factories

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Lignocellulose biorefinery is an integral part in bio- and circular economy. However, efficient use of lignocellulose is hindered as lignin, a major component of lignocellulose, is underexploited due to its recalcitrance and inherent heterogeneity. Although some strategies for lignin depolymerization have been studied, the depolymerization of lignin gives rise to various heterogeneous aromatic compounds, making it challenging for lignin valorization. This study aims to build novel cell factories that utilize these lignin-derived molecules (LDMs) as substrate to produce high value products, wax ester and alkane. As an excellent organism for metabolic engineering, Acinetobacter baylyi ADP1 serves as the platform for the cell factory construction. A. baylyi ADP1 can funnel various LDMs into central metabolite, acetyl-CoA, through β-ketoadipate pathway and naturally synthesize wax ester as storage compound. The strain is also engineered for alkane synthesis. Metabolic engineering is employed to optimize substrate consumption and the production of wax ester and alkane.

P55

Product diversification helps to add value in the chemithermomechanical processing of sugarcane bagasse

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Alkaline-sulfite chemothermomechanical (CTMP) processing of sugarcane bagasse proved an efficient way to decrease biomass recalcitrance. However, economic evaluation of this process, integrated to a first generation autonomous ethanol mill, indicates internal return rates highly dependent on the chemical costs. In this scenario, product diversification was exploited to overcome economical limitations of the integrated process. Despite pretreated solids can be efficiently converted to a glucose and xylose rich syrup, xylan extraction prior to enzymatic hydrolysis showed a suitable step to produced diverse xylan molecules depending on the extraction procedure. Pretreatment liquor was also used to re-sulfonate residual lignin, increasing lignosulfonate production in the process. Hydroxycinnamates were also mapped along the process indicating some alternative routes to recover these antioxidants in the biorefinery concept. Mass balance for entire process and structural characteristics of each fraction suggests that product diversification is feasible in CTMP-based biorefinery.

P56

Use of agroindustrial residues as raw material for production of selenium-enriched Kluyveromyces marxianus biomass

Sabrina Evelin Martiniano[1], Rafael Rodrigues Philippini[1], Júlio César Santos[1], **Silvio Silverio da Silva**[1]

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Se-enriched biomass produced from lignocellulosic residues is an interesting and inexpensive alternative for animal feed. Selenium has an important role in animal's health, growth and fertility. The present work deals with the production of Kluyveromyces marxianus biomass enriched with selenium for animal feed, using sugarcane bagasse hemicellulosic hydrolysate (SBHH) as raw material. SBHH was prepared by acid hydrolysis and the fermentation was performed in Erlenmeyer flasks with non-detoxified hydrolysate, supplemented with 15 mg/L Na2SeO3. After 72 hours of fermentation, a concentration of 1.4 g/L of biomass was obtained, with 87 % viability and enriched with 8298 ppm of selenium. Only 28 % of sugars consumption was observed, probably due to toxic compounds in hydrolysate, indicating future studies of hydrolysate detoxification. Potential of use of SBHH for Se-enriched biomass production was demonstrated, this proposal representing an alternative for integration in a biorefinery context.

P57

A novel activity of the Chromobacterium violaceum ω-transaminase yielding aminated galactose-substituted carbohydrates

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Oligosaccharide amines have potential as monomers in the synthesis of novel biopolymers. We used a Chromobacterium violaceum ω-transaminase, known for its broad substrate range, to utilize sugar aldehydes as amine acceptors: galactose, raffinose and galactose-containing xyloglucan oligosaccharides, with the galactose residues in each substrate containing an aldehyde group on carbon C-6 instead of a hydroxyl. These amine acceptors were produced by regioselective oxidation of the C-6 hydroxyl by a Fusarium graminearum galactose oxidase. Successful amination of (mono) galactose by the ω-transaminase with two different amine donors ((S)-(−)-α-methylbenzylamine and isopropylamine) was confirmed by LC-Q-TOF MS and HPAEC-PAD. Amination of the galactose residue of raffinose was confirmed by MALDI-TOF. Moreover, results from a spectrophotometric transaminase activity assay show activity towards galactoxyloglucan oligosaccharides. This was the first demonstration of transaminase activity towards oligosaccharides.

P58

Functional Polymeric Materials with very High Levels of Four Unmethylated Lignins

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For 60 years, the hydrodynamic compactness of macromolecular lignin species was incorrectly ascribed to crosslinking between lignin chain segments. As a result, functional polymeric materials with very high lignin contents emerged only recently. Thus, 2–5 wt% proportions of compatible blend components can lead to softwood ball-milled lignin-based materials that exceed polystyrene in tensile strength. Softwood ligninsulfonates are more challenging in as far as 15 wt% blend-component levels are required in materials that surpass the tensile behavior of polystyrene. Otherwise, materials containing 95 wt% industrial kraft lignin are capable of exceeding the tensile strength of polyethylene, and similar behavior is encountered with materials composed solely of maple γ-valerolactone lignin. In some cases, the properties of lignin–lignin blends embody dramatic improvements over materials produced from the predominant lignin preparation alone. The foregoing observations are consistent with a single working hypothesis that describes how material continuity in these new lignin-based plastics is achieved.

P59

Production of lipids from glucose, xylose and mannose by oleaginous yeasts

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The yeast lipids are a resource with potential applications in nutritional food, biofuel and oleochemical industry. Recent studies have focused on the search of substrates and yeasts for a viable production of lipids. In this study, it was proposed the lipids production through Scheffersomyces coipomensis, Sugiyamaella paludigena y Meyerozyma guilliermondii, using glucose, xylose and mannose as substrates. The yeasts were cultured using 40 g/L monosaccharides. Biomass and lipids were determined in dry cells. Further the lipid coefficients in culture medium and by consumed substrates were calculated. The results showed that the three yeasts grew in the monosaccharides and a percentage of them were converted into lipids. S. coipomensis had the highest biomass yield and lipid production with 14.6 g/L and 39% w/w on mannose respectively with productivities were 0.05 g/L/h and 15.4 g/100g. Information not previously reported. This investigation proposes a potential application of the forest biomass for lipid production.

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Novel applications for traditional lignocellulose pretreatments

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The application field for biorefinery technologies developed for 2nd generation biofuels is expanding towards higher value products and novel raw materials. Grasses and bran both comprise a lignocellulosic fibre fraction, combined with protein and free sugars or starch. Grasses are high-yield high-protein feed crops with surplus production capacity, while bran is a side stream of cereal industry with limited food applications. In the present work, the aim was to process grass silage fibre and wheat bran using steam explosion and alkaline treatment, which are among the well-studied pretreatments facilitating enzymatic hydrolysability of lignocellulosic carbohydrates. Grass silage was processed into protein extract and lignocellulosic sugars, which were fermented into single cell protein for feed applications. Wheat bran was processed into functionalized sugar syrup as a novel food industry ingredient, high in prebiotic arabinoxylo-oligomers and antioxidants. This research illustrates the potential of biorefinery technologies for production of valuable food and feed components.

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Evaluation of chemical modified kraft lignin as depressant of molybdenite

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Molybdenite is a co-product obtained from copper mining and kraft lignin recovered from Pinus radiata black liquor was modified by sulfomethylatikon reaction with the aim to increase its sulfonation degree for use as molybdenite depressing agent. An experimental design of the sulfomethylation process was performed by using temperature, time and sodium sulfite as factors followed by characterization of the lignin products. Results showed that the interaction of time, temperature and sodium sulfite concentration had an effect on the depression of molybdenite by modified lignin (p-value=0.001, R2=0.92). After lignin sulfomethylation, an increase in SO3 groups (2.2 mmol/g) and in OH amount was observed. The ability of sulfomethylated lignin to depress molybdenite was significantly higher (93.2%) than commercial calciuym lignosulfonate (59.2%) Therefore, the sulfomethylation of kraft lignin could have a potential application in the mining industry as a mineral depressant.

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Biosurfactant production by yeast from lignocellulosic biomass

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Biosurfactants are microbial metabolites with surfactants, emulsifiers, low toxicity and high biodegradability properties, that have the possibility of wide application in different industries. The present work shows biosurfactants production from sugar cane bagasse hemicellulosic hydrolyate as an option of bioproduct in future lignocellulosic biorefineries. The experiments were conducted with Cutaneotrichosporon mucoides according to a 2^2 full factorial design, in 500 mL Erlenmeyer flasks containing different medium volumes (50 mL, 150 mL and 250 mL) based on detoxified sugarcane bagasse hydrolysate with approximately 40 g/L of xylose and supplemented with nutrients. Different combination of agitation (100, 175 and 250 rpm) and ratio medium volume of 0.1, 0.3 and 0.5 were used. The best results (68.8 % of EI and 52.5 $mN.m^{-1}$ of ST) were obtained in the fermentation with the highest agitation level (250 rpm) and the highest medium volume (250 mL).

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Cellulose Defibrillation into Cellulose Microfibrillated and Cellulose Nanofibrillated by Ultra-Refining: A Kinetic Study

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Cellulose microfibrillated (CMF) and nanofibrillated (CNF) can be applied in a broad range of products. Their potential applications arise primarily from their intrinsic properties enhanced and/or gained during defibrillation of cellulose fibers into micro and nanofibrils. To understand how the fiber properties changes during the isolation of CMF and CNF, the kinetic of the ultrarefining of cellulose in a disc ultra-refiner was performed and the properties of the materials characterized. Most changes in properties (i.e. crystallinity, viscosity, transparency) during defibrillation were observed up to isolation of CMF, a heterogeneous suspension with microfibrils and fragments of fibers. Further processing enhanced defibrillation, yielding CNF with different diameters, and therefore different aspect ratios, but with similar physical properties and also with better uniformity of the particles diameters. Detailed kinetic study allowed for more precise evaluation and selection of the degree of defibrillation suitable to obtain materials for specific application while minimizing energy consumption.

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Intensification approach for biomolecules production from lignocellulosic materials

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In this work, we present a promising Intensification approach for bioethanol production by using sugarcane bagasse (SB) as a carbon source, by using reduced numbers of equipments. Initially, SB was submitted to alkaline hydrolysis in 250 ml Erleymeyer flask (0.5M NaOH; 90ºC; 4h; 0.04 w/v), to reduce lignin content and hence, increasing enzymatic digestibility. After this process, solubilized lignin was removed and remaining portion of holocellulose was kept and washed in the same flask. Thus, in a process of simultaneous sacharification and fermentation (citrate buffer+ 20 FPU of cellulase/g of biomass for 48h at 50ºC, and, addition of 0.5 g/L of cells of *Sheffersomyces shehatae* UFMG-HM 52.2 + nutritional media for 72h at 30ºC) was performed. Ethanol yield of 0.42 g/g of sugars (xylose+glucose) and productivity of 0.40 g/L.h were verified. Results highlighted the feasibility of the research proposal, indicating further studies in different bioreactors aimed to scale-up the bio-process.

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Direct evidence for the contribution of ray cells to tracheid lignification in developing Norway spruce xylem

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In the lignifying xylem of Norway spruce, a comparative transcriptomic study has suggested that parenchymal ray cells contribute to monolignol biosynthesis used for cell wall lignification of tracheids; however, no metabolic information has been obtained at the single cell level. In this work, a newly developed in-situ single cell metabolomics, picolitre pressure-probe-electrospray-ionization mass spectrometry (picoPPESI-MS), was performed to directly determine the metabolites in the cellular fluids individually collected from both upright tracheids and parenchymal ray cells in developing xylem of semi-intact young plants. The picoPPESI-MS results showed that monolignols and their glycoconjugates were present in both cell types, with some cell-to-cell variations in the composition, indicating that the biosynthetic route for monolignols was active in each cell type. Hence, our data strongly support the hypothesis that adjacent parenchymal ray cells produce monolignols and participate in lignification of tracheid cell walls.

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