9th PhD Conference Tulln

5th and 6th October 2022

Organized by the Institute of the Environmental Biotechnology (UT)

Universitäts- und Forschungszentrum Tulln

Konrad-Lorenz-Straße 24

3430 Tulln

Book of abstract

Oral Presentation

Giorgio Licciardello

Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Italy

giorgio.licciardello@unitn.it

Enhancement of plant tolerance to cold stress by Antarctic psychrotolerant bacteria

---

Giorgio Licciardello[1,2,3], Maria Doppler[3], Alexandra Parich[3], Rainer Schuhmacher[3], Michele Perazzolli[1,2]

[1] Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Italy;
[2] Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Italy;
[3] Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology (IFA-Tulln),University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Straße 20,3430 Tulln, Austria.

The use of bacteria associated with Antarctic plants may represent a sustainable strategy for crop protection against cold stress. However, scarce information is available on the mechanisms underlying this process. This work aims at understanding the mechanisms activated by psychrotolerant bacteria on tomato plants and to identify metabolites involved in the mitigation of cold stress. Two bacteria isolated from the Antarctic plant *Colobanthus quitensis* (Hafnia sp. B7 and *Pseudomonas sp. A3*) were able to colonize tomato seedlings promoting shoot growth at low temperatures. Surface-disinfected tomato seeds were inoculated with psychrotolerant bacteria or mock-inoculated as a negative control. Four-week-old plants were exposed to 4°C for 7 days and incubated at 25°C for zero, two and four days to allow the recovery. The content of polyphenols and amino acids was determined using liquid chromatography (LC) and hydrophilic interaction liquid chromatography (HILIC) in combination with high resolution mass spectrometry analysis (HRMS), respectively. Chlorogenic acid, p-coumaric acid, rutin, ferulic acid and all the amino acids were detected in tomato samples. The content of p-coumaric acid increased upon cold stress and decreased during the recovery in mock-inoculated plants, while the content of chlorogenic acid, ferulic acid and phenylalanine increased during the recovery. The isolate A3 tended to reduce the proline content after cold stress and during recovery, suggesting that it may affect proline metabolism. The effects of A3 and B7 on the tomato metabolic content are in progress. The role of these compounds in the cold tolerance of tomato plants will be analysed by functional analyses. Moreover, a tracer phenylalanine labelled with 13C will provide insights on the phenylalanine-derived compounds (such as flavonoids) to better understand their contribution in the mechanism of cold tolerance. The study will provide a better knowledge on the cold tolerance mechanisms activated by beneficial bacteria in tomato plants to further develop bacterial-based strategies against cold stress.

Kangkang Xu

University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln), Christian Doppler Laboratory for Innovative Gut Health Concepts of Livestock, Konrad-Lorenz-Str. 20, 3430 Tulln, Austria

kangkang.xu@boku.ac.at

Comparison of chromatographic conditions for the targeted tandem mass spectrometric determination of 344 mammalian metabolites

Kangkang Xu[1], Dimitrios J. Floros[1], Franz Berthiller[1], Heidi Schwartz-Zimmermann[1]

[1] BOKU-University of Natural Resources and Life Sciences, IFA Tulln, Austria

Metabolomics, the process of measuring a wide range of small molecules in any biological system, has become an increasingly popular "omics" approach in biomedical research and nutritional analysis [1]. Comparatively, the use of modern metabolomics technology in livestock research is still lagging behind [2]. To develop a set of targeted multi-analyte LC-MS/MS methods for metabolite quantification and biomarker discovery, we constructed a metabolite library with 344 mammalian metabolites from 19 compound classes, including sugars, amino acids, carboxylic acids, nucleotides and various lipid classes. We then optimized multiple selected reaction monitoring transitions for each compound on a triple quadrupole mass spectrometer. Subsequently, we compared the retention profiles of our metabolite library across different chromatographic conditions: three reversed-phase (RP) methods (C18, F5, C18 under lipidomics conditions), three hydrophilic interaction liquid chromatography (HILIC) methods (bare silica-, zwitterionic-based HILIC, zwitterionic-based HILIC at pH 9) as well as anion exchange chromatography (AIC). HILIC methods covered on average 54% of the library metabolites with retention factor >1 and symmetrical peak shape, while the RP method coverage was < 45%. In contrast to RP, HILIC methods could also retain polar metabolites such as amino acids and biogenic amines. Carboxylic acids, nucleotides, and sugar related compounds were predominantly targeted by AIC or zwitterionic pHILIC at alkaline pH. Combining zwitterionic pHILIC at alkaline pH with the complementary RP-F5 method covered 91% of the library metabolites, but failed to target short chain fatty acids. The combination of bare-silica HILIC, RP-F5 and AIC achieved the highest total library coverage of 97%. This extensive survey of both chromatographic and MS properties provides a diverse and comprehensive dataset and will facilitate the development of quantitative targeted LC-MS/MS methods for livestock metabolomics.

References

[1] Wishart, D.S., *Metabolomics: applications to food science and nutrition research.* Trends in food science & technology, 2008. **19**(9): p. 482-493.

[2] Goldansaz, S.A., et al., *Livestock metabolomics and the livestock metabolome: A systematic review.* PLOS ONE, 2017. **12**(5): p. e0177675.

Acknowledgements

This research was performed in the framework of the Christian Doppler Laboratory for Innovative

Gut Health Concepts of Livestock, funded by the Austrian Federal Ministry for Digital and Economic Affairs, the National Foundation for Research, Technology and Development and by BIOMIN Holding GmbH, which is part of DSM.

Mara Meisenburg

Laboratory of Plant physiology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, Netherlands

mara.meisenburg@wur.nl

Light spectral composition impacts defense related metabolome in tomato plants

Mara Meisenburg[1], Mark Veen[1], Marijke D. Haas[1], Selina Ilchmann[1], Francel Verstappen[1], Alexander R. van der Krol[1], Iris F. Kappers[1]

[1] Laboratory of Plant Physiology, Wageningen University, The Netherlands

In the Northern hemisphere, tomato is mostly grown in greenhouses where they still risk exposure to different pathogens, fungi or herbivores. To protect themselves against biotic stressors, the plants produce secondary metabolites, which are influenced by light quality in a growth-defense trade-off. In this trade-off, the negative influence of shade (relatively rich in Far Red light) has been researched extensively. Here, we compared the effect of added blue, red, or far-red light on the default tomato defense-related metabolome. Five-week-old tomato plants were exposed for five days to different experimental light conditions: white (W) light (control), supplemented W with blue (B), with red (R) and far-red (FR), while photosynthetic active radiation was kept the same for all conditions. After five days light treatment, plant morphology was determined and young and old leaves were sampled at the end of the photoperiod or end of dark period. FR treated plants showed increased elongation, and B and FR treated plants had increased fresh weight compared to WT and R exposed plants. Leaf samples were used to measure primary and secondary metabolites. Results showed that soluble sugars were reduced in tomato plants exposed to B, while anthocyanins, □-tomatine and its precursor tomatidine were reduced by B and FR. Under R abundances of these metabolites remained like in plants grown under W. Untargeted analysis for extracted endogenous metabolites displayed a similar clustering as result of light conditions, though it was clearer seen in the young leaves than in the old ones. Headspace analysis of emitted volatiles showed effects of light treatment and trapping timepoint. Overall, results revealed that light conditions affect plant metabolome at various levels. Since effects on different defense related compounds varied in different directions, it remains difficult to infer overall impacts on plant defense potential.

Acknowledgements

'LEDs make it resilient' is a NWO Green II financed project (ALWGR.2017.004). Enza Zaden, Biobest, Florensis, Schoneveld Breeding, WPK, and Heliospectra are partners of the consortium. I would further like to thank Jonathan Samson for sharing his R script.

Katharina Gasser

Institute of Plant Protection, Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz-Straße 24, 3430 Tulln an der Donau, Austria

katharina.gasser@boku.ac.at

Fusarium in garlic: species diversity and mycotoxin production

Katharina Gasser[1], Michael Sulyok[2], Bernhard Spangl[3], Rudolf Krska[2], Siegrid Steinkellner[1], Karin Hage-Ahmed[1]

[1] Institute of Plant Protection, Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna
[2] Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Vienna
[3] Institute of Statistics, Department of Landscape, Spatial and Infrastructure Sciences, University of Natural Resources and Life Sciences, Vienna

Fusarium proliferatum and *F. oxysporum* are worldwide known pathogens of garlic. While *F. proliferatum* causes the so-called dry rot, *F. oxysporum* is considered as causal agent of basal rot [1]. *F. proliferatum* is known for the production of fumonisins [2]. The aim of this study was to identify the Fusarium species in Austrian garlic and to evaluate the profile of the secondary metabolites produced *in vitro*. In addition, commercial garlic was examined for contamination with these metabolites. For this purpose, representative *Fusarium* isolates were selected, collected over two growing seasons from seed and stored garlic as well as from two classic market varieties. The majority of the isolates were identified as *F. proliferatum*. All remaining isolates were identified as *F. oxysporum*. Almost all isolates of *F. proliferatum* selected for secondary metabolite production *in vitro* produced fumonisins. Statistical analysis revealed that the garlic variety, respectively its geographical origin had a significant influence on the secondary metabolite profile of the *F. proliferatum* isolates. In contrast to the frequent production of fumonisins *in vitro*, these mycotoxins were detected in commercial garlic only sporadically and in very low amounts. In conclusion, this study identified *F. proliferatum* as the main pathogen in Austrian seed and commercial garlic. Despite the proven production of fumonisins *in vitro*, these mycotoxins were detected in commercial garlic only in small amounts.

References

[1] Schwartz, H. F., and Mohan, S. K. 2008. Compendium of onion and garlic diseases and pests. Second edition. The disease compendium series. American Phytopathological Society, St. Paul, MN.
[2] Desjardins, A. E. op. 2006. Fusarium mycotoxins: Chemistry, genetics, and biology. The American Phytopathological Society, St. Paul, Minnesota.

Acknowledgements

We thank Marc Lemmens for kindly providing Fusarium strains IFA22 and IFA350 as well as Karin Baumgartner and Tina Austerlitz for their excellent technical support. This work was partially supported by the Austrian Federal Ministry of Agriculture, Forestry, Regions and Water Management and the European Union (EIP-project „Bodenbürtige Krankheiten im Knoblauchanbau“).

Sophia Mihalyi

Institute of Environmental Biotechnology, IFA-Tulln, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Straße 20, 3430 Tulln an der Donau, Austria

sophia.mihalyi@boku.ac.at

Simultaneous recovery of pure polyester and production of valuable lactic acid from blended textile waste

Sophia Mihalyi[1], Felice Quartinello[1,2], Georg Guebitz[1,2]

[1] *Institute of Environmental Biotechnology, IFA-Tulln, Department of Agrobiotechnology, University of Natural Resources and Life Sciences Vienna, Konrad Lorenz Straße 20, 3430 Tulln an der Donau, Austria*

[2] Austrian Centre of Industrial Biotechnology, Konrad Lorenz Strasse 20, 3430 Tulln an der Donau, Austria

The generation of textile waste represents a serious challenge nowadays since fast fashion concepts lead to an increase of consumption and decrease of life span of clothing. On average Europeans discard 11 kilograms of textiles per person and year and 87 % of the textile waste is still landfilled or incinerated [1, 2]. Therefore, circular instead of linear models must be developed including collection and recycling approaches. Textiles usually consist of blended fibers which represents a challenge in recycling processes. Textile blends comprise natural and synthetic fibers such as for example cellulose (cotton, viscose) and polyester, respectively. Biotechnological approaches enable the specific separation of fibers by the application of enzymes. The cellulosic fibers can specifically be hydrolyzed by cellulases leaving the synthetic polyester (PET) fibers as pure material for textile-to-textile recycling thereby saving valuable resources [3]. The recovered building block glucose from the depolymerization of cellulose can be used as a substrate in microbial fermentation processes for valorization of each component. To further decrease the consumption of energy and resources in such a recycling approach, the concept of simultaneous saccharification and fermentation (SSF) is introduced in this study. The intermediate steps of recovery and separation of the glucose solution are not required in the SSF process. The bacterium *Weizmannia coagulans*, that can grow at the same conditions as the enzymatic hydrolysis is performed, was deployed for the SSF approach. This organism produces lactic acid that can further serve as a building block for synthesis of the bio-based polylactic acid (PLA) [4]. In this concept two valuable products, pure PET and lactic acid, can be recovered from one process, valorizing each major component of blended textile waste consisting of cellulosic fibers and polyester, to save resources, reduce greenhouse gas emissions and support the development towards a circular economy.

References

[1] The impact of textile production and waste on the environment (infographic), European Parliament, 2020.

[2] Textiles in Europe's circular economy, European Environment Agency, 2019.

[3] F. Quartinello, S. Vecchiato, S. Weinberger, K. Kremenser, L. Skopek, A. Pellis, G.M. Guebitz, Highly Selective Enzymatic Recovery of Building Blocks from Wool-Cotton-Polyester Textile Waste Blends, Polymers 10(10) (2018) 1107.

[4] J. Li, S. Shi, Y. Wang, Z. Jiang, Integrated production of optically pure l-lactic acid from paper mill sludge by simultaneous saccharification and co-fermentation (SSCF), Waste Management 129 (2021) 35-46.

Acknowledgements

This project has received funding from the Horizon 2020 Programme under grant agreement n°101003906.

Chiara Siracusa

Institute of Environmental Biotechnology, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, IFA-Tulln

Chiara.siracusa@groupwise.boku.ac.at

A step forward in enzymatic depolymerization of polyesters: generation of highly pure monomers

Chiara Siracusa[1], Felice Quartinello[1,2], Alessandro Pellis[3], Georg M Guebitz[1,2]

[1] acib GmbH, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria.
[2] Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences Vienna Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria.
[3] Università degli Studi di Genova (UNIGE), Department of Chemistry and Industrial Chemistry

Besides mechanical and chemical methods, polyester recycling has been recently addressed with enzymatic treatment. This would allow to overcome the main issues of the classical approach, namely the difficult contaminants separation or the poor quality of the recycled products. Pressing and heating may damage the material. Moreover, the process could result in pollutant fumes as side products [1]. The focus of this work is different kinds of plastics: polyethylene terephthalate (PET), Polybutylene adipate co-terephthalate (PBAT) and some blended biopolymers such as PBAT/Starch. *Humicola insolens* cutinase (commercially available cutinase) was applied for the hydrolysis of the above-mentioned polyesters. Released soluble monomers were quantitatively monitored through High-Performance Liquid Chromatography (HPLC).
Terephthalic acid (TPA) is a versatile and common component of both PBAT and PET. It was initially separated from post-hydrolysis solutions through acidification and centrifugation. The solid was then characterized in purity via Fourier Transform-Infrared and ^{1}H-NMR analysis. While TPA obtained from PET proved to be 95% pure, the one from PBAT still presented 15% of other monomers on the total. Adipic acid and 1,4-Butanediol are in fact similarly affected by same pH-shift. Further steps were then necessary to increase final purity. Remaining PET hydrolysate contains Ethylene glycol. Its isolation from the residual fraction, as well as that of Adipic acid and 1,4-butanediol from PBAT, was investigated. Resynthesis and microbial fermentation [2] can exploit these monomers only with a low presence of contaminants. Enzymatic hydrolysis of plastics is therefore extremely promising not only in terms of environmental-friendly processing, but also for the recovery of monomers at high quality level. These could re-enter production cycle with same grade of virgin material. The applied methods in particular allows a straightforward handling and prove to be economically competitive. This aspect cannot be disregarded in the context of the current preferred follis based plastic production [3].

References

1. Maurya, A., Bhattacharya, A., & Khare, S. K. (2020). Enzymatic Remediation of Polyethylene Terephthalate (PET)–Based Polymers for Effective Management of Plastic Wastes: An Overview. Frontiers in Bioengineering and Biotechnology, 8(November), 1–13. https://doi.org/10.3389/fbioe.2020.602325
2. Tiso T, Winter B, Wei R, et al. The metabolic potential of plastics as biotechnological carbon sources - Review and targets for the future. Metab Eng. 2022;71:77-98. doi:10.1016/j.ymben.2021.12.006
3. Lee, H. L., Chiu, C. W., & Lee, T. (2021). Engineering terephthalic acid product from recycling of PET bottles waste for downstream operations. Chemical Engineering Journal Advances, 5(October 2020), 100079. https://doi.org/10.1016/j.ceja.2020.100079

Katharina Steiner

Department of Agrobiotechnology, University of Natural Resources and Life Sciences Vienna, 3430 Tulln an der Donau, Austria
Wood K plus – Kompetenzzentrum Holz GmbH, 4040 Linz, Austria

k.steiner@wood-kplus.at

Challenges in the enzymatic recycling of blended textiles

Katharina Steiner [1,2], Felice Quartinello[2], Doris Ribitsch [2,3]

[1] Wood K plus – Kompetenzzentrum Holz GmbH, Linz, Austria
[2] Institute for Environmental Biotechnology, IFA Tulln, University of Natural Resources and Life Sciences, Vienna, Austria
[3] ACIB – Austrian Centre of Industrial Biotechnology, Graz, Austria

The end-life for clothes is decreasing, which corresponds to a tremendous production rate of textiles. Globally, the majority of end of use textiles is landfilled or combusted, less than 1 % is reprocessed in a recycling loop [1]. However, reprocessing of fiber blends remains a challenge as currently available technologies are not efficient enough. One potential approach to close the recycling loop is to degrade the minor component of the textile enzymatically and further reprocess the major component to new fibers. For that, Cellulases are used to hydrolyse the cellulosic component, whereas cutinases are used to eliminate the polyesterfraction. To maximise degradation yield, inherent pitfalls of enzyme and substrate need to be identified and remedied. In the case of cellulases respectively cellulose as corresponding substrate, product inhibition as well as high substrate crystallinity are generally known to decrease cellulose digestability [2]. Product inhibition is a mechanism which prevents the enzyme of metabolizing more substrate. Crystallinity describes the highly ordered structure of cellulose fibrils. It is associated with the formation of new hydrogen bonds that provide a kind of resistance to chemical and biological depolymerisation [3]. These two effects have been demonstrated within this study. Therefore, alkaline pretreatment of blended textiles was attempted to amorphise the cellulose and increase its conversion rate. Both product inhibition and high substrate crystallinity were shown to influence degradability. Alkaline pretreatment can be used to counteract the high crystallinity. However, circumvention of the product inhibition is only realisable with a suitable reactor design.

References

[1] Textile Exchange. (2021). Preferred Fiber & Material, Market Report 2021. https://textileexchange.org/wpcontent/uploads/2021/08/Textile-Exchange_Preferred-Fiber-and-Materials-Market-Report_2021.pdf.
[2] Geboers, Jan A.; van de Vyver, Stijn; Ooms, Roselinde; Beeck, Beau op de; Jacobs, Pierre A.; Sels, Bert F. (2011): Chemocatalytic conversion of cellulose: opportunities, advances and pitfalls. In: Catal. Sci. Technol. 1 (5), S. 714. DOI: 10.1039/c1cy00093d.
[3] Ling, Zhe; Chen, Sheng; Zhang, Xun; Xu, Feng (2017): Exploring crystalline-structural variations of cellulose during alkaline pretreatment for enhanced enzymatic hydrolysis. In: Bioresource technology 224, S. 611–617. DOI: 10.1016/j.biortech.2016.10.064.

Acknowledgements

The authors would like to thank the consortium of the EnzATex research project led by Transfercenter

für Kunststofftechnik GmbH involving the following project partners: Erema Group GmbH, IFG Asota GmbH, i-RED Infrarot Systeme GmbH, Kompetenzzentrum Holz GmbH, Lenzing Aktiengemeinschaft, Linz Textil Gesellschaft m.b.H. O.Ö. Landes-Abfallverwertungsunternehmen GmbH, PROFACTOR GmbH and Tiger Coatings GmbH. A special thanks goes to Lenzing Aktiengemeinschaft for providing fiber material. A further thanks needs to be addressed to Elmar Janser at Novozymes who kindly provided the enzyme formulations.

Miguel Jimenez Bartolome

Department of Agrobiotechnology, University of Natural Resources and Life Sciences Vienna, 3430 Tulln an der Donau, Austria

miguel.jimenez-bartolome@boku.ac.at

Improving the properties of starch-based adhesives using laccase polymerized lignosulfonate

Miguel Jimenez Bartolome[1], Nikolaus Schwaiger[2], Rene Flicker[3], Bernhard Seidl[3], Martin Kozich[3], Gibson S.Nyanhongo[1], Georg Guebitz[1]

[1] Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 20, 3430, Tulln an der Donau, Austria
[2] Sappi Papier Holding GmbH, BruckerStrasse 21, 8101, Gratkorn, Austria
[3] Agrana Research & Innovation Center GmbH, Josef Reitherstraße 21-23, 3430 Tulln, Austria
[4] University of Johannesburg, Department of Biotechnology and Food Technology, Faculty of Science, Corner Siemert and Louisa, Doornfontein 2028, John Orr Building, Johannesburg, South Africa.

The second most abundant biopolymer in nature is lignin although it is underexploited and mainly burned for energy generation. Only about 2% of the annually produced lignin is currently used in value-added applications. Enzymatic oxidation of lignosulfonates (LS) can generate a variety of value-added products, like coatings, binders, adhesives, hydrogels among several others. A novel strategy for improving wet resistance and mechanical properties of starch-based adhesives using enzymatically polymerized LS as additives was developed. Starch-based adhesives are highly water-soluble, limiting applicability in the industry. Incorporation of laccase polymerized LS increased the wet resistance of the adhesives. The starch-based adhesives supplemented with highly polymerized LS (>4500 kDa) led to a great increase on the wet resistance (from 15 to 20 minutes or from 150 to 1200 minutes depending on the type of starch-adhesive) while, at the same time, not affecting, or even improving the bonding time. The addition of different amounts of polymerized LS to the starch-based adhesive indicated that there is a sensitive balance point between improved wet resistance and negative effects on bonding time. Using laccase inhibitors, it was demonstrated that the presence of an active enzyme is important for the beneficial development of the properties of the adhesive. This study therefore shows that the addition of the proper amount of enzymatically polymerized LS to starch-based adhesives is a highly viable strategy for improving their mechanical properties, especially the wet resistance.

Book of abstract

Posters

1. Cicely Warne

Institute of Environmental Biotechnology, Department of Biomaterial and Enzyme Technology, University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau

cicely.warne@boku.ac.at

Greener Enzymatic Synthesis of Levoglucosenone-based Polyesters

Cicely M. Warne[1], Sami Fadlallah[2], Florent Allais[2], Georg M. Guebitz[1, 3], Alessandro Pellis[3, 4]

[1] Austrian Centre of Industrial Biotechnology (ACIB), Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria.
[2] URD Agro-Biotechnologies Industrielles (ABI), CEBB, AgroParisTech, Pomacle 51110 France
[3] Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria.
[4] Università di Genova, Dipartimento di Chimica e Chimica Industriale, via Dodecaneso 31, 16146, Genova (GE), Italy

With fossil fuels depleting fast, society needs to find alternatives to the current oil-based plastics. The obvious solution is to utilize biomass as a source of carbon, as much of it is currently discarded as waste. Many bio-derived platform molecules are being used to synthesize polymers, one of which is levoglucosenone (LGO). Derived from cellulose feedstock such as wood pulp[1], it is a very versatile organic molecule with functionalized chiral structure. This makes it a useful molecule in many reactions, such as in the synthesis of the solvent Cyrene[1]. There have been several previous works that focus on LGO -based polymers[2–4], however, these methods used traditional metal catalysts or non-green reagents. Enzymes are a more sustainable alternative; they are inherently bio-based and often require mild reaction conditions. Here, the polymerization of several LGO -derived monomers (synthesised according to previous works[2-3,5]), 2H-HBO-HBO, HO-HBO and triol citro, was attempted with the biocatalyst Candida antarctica lipase B (CaLB) in several green solvents.
The yields for Poly(2H-HBO-HBO adipate) were initially very low. Using a less polar anti-solvent such as 2,2,5,5-tetramethyloxolane (TMO) instead of water resulted in a much better yield, up to 81%. TMO is considered a green solvent, and the yield is much improved compared to the previous work of 38%[3]. We have also managed to produce poly(HO-HBO adipate) at a good yield using an enzymatic polycondensation reaction. Yield and molecular weight are similar to previously published results[2], but this method is greener as it substitutes diesters for diacyl chlorides. Finally, triol citro has also been polymerised using enzymes. It was estimated that use of an enzyme would decrease branching, and produce a linear polymer. Several polymers have been produced, and branching is estimated to be minimal, while achieving molecular weights of up to 8 kDa.

References

[1] J. E. Camp, ChemSusChem, 2018, 11, 3048–3055.

[2] F. Diot-Néant, L. M. M. Mouterde, J. Couvreur, F. Brunois, S. A. Miller and F. Allais, European Polymer Journal, 2021, 159, 110745.
[3] F. Diot-Néant, L. Mouterde, S. Fadlallah, S. A. Miller and F. Allais, ChemSusChem, 2020, 13, 2613–2620.
[4] S. Fadlallah, A. L. Flourat, L. M. M. Mouterde, M. Annatelli, A. A. M. Peru, A. Gallos, F. Aricò and F. Allais, Macromolecular Rapid Communications, 2021, 42, 2100284.
[5] A. Kayishaer, S. Fadlallah, L. M. M. Mouterde, A. A. M. Peru, Y. Werghi, F. Brunois, Q. Carboué, M. Lopez and F. Allais, *Molecules*, 2021, **26**, 7672.

Acknowledgements
This work was supported by European Union's Horizon 2020 research and innovation program under grant agreement No 953073, project UPLIFT (sUstainable PLastIcs for the Food and drink packaging indusTry).

2. Filippo Fabbri

Department of Agrobiotechnology, Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 20, 3430 Tulln an der Donau, Austria

filippofabbri@groupwise.boku.a.at

Elucidating Enzymes Selectivity and Thermostability in Short-Esters and Polyesters Synthesis

Fabbri Filippo[1,2], Bertolini A. Federico[1], Guebitz M. Georg[1,2], Pellis Alessandro[1,3]

[1] BOKU-University of Natural Resources and Life Sciences, IFA Tulln, Austria
[2] Austrian Centre of Industrial Biotechnology, IFA Tulln, Austria
[3] University of Genoa, Department of Chemistry and Industrial Chemistry, Genoa, Italy

Various bio-based aliphatic building blocks, such as adipic acid, itaconic acid, sebacic acid and 1,4-butandiol were investigated for the biocatalyzed synthesis of polyesters [1]. Different hydrolases, namely *Candida antarctica* lipase B (CaLB), *Humicola insolens* cutinase (HiC), and the in-house produced *Thermobifida cellulosilytica* cutinase 1 (Thc_Cut1) [2,3] were successfully adsorbed onto polypropylene beads [4] and evaluated using a full-factorial design of experiments (DoE) for the synthesis of short-esters. The activity of the biocatalysts in transesterification was screened in a broad range of temperatures (50-90 °C) considering different carbon chain lengths for alcohols and acids (C_4, C_8 and C_{12}). The DoE allowed to obtain a 4D contour response, which indicated CaLB, HiC and Thc_Cut1 temperature optima at 85 °C, 70 °C, and 50 °C, respectively. Furthermore, CaLB and HiC exhibited their selectivity towards long-chain alcohols and acids as substrate in contrast to Thc_Cut1, which was more active on short-chain monomers. Finally, the obtained model was verified by biocatalyzed synthesis of polyesters through polycondensation reactions, using the previously investigated immobilized hydrolytic enzymes. CaLB and HiC showed the best synthetic activity when used with dimethyl sebacate (DMSe) while a decrease in polymers M_w was observed with dimethyl adipate (DMA) and dimethyl itaconate (DMI). On the other hand, Thc_Cut1 led to polyesters with lower molecular masses, despite the high conversion rate achieved with DMA. These results fully confirmed our model for all of the three enzymes.

References

[1] Pellis, A., Corici, L., Sinigoi, L., D'Amelio, N., Fattor, D., Ferrario, V., ... & Gardossi, L. (2015). Towards feasible and scalable solvent-free enzymatic polycondensations: Integrating robust biocatalysts with thin film reactions. *Green Chemistry*, *17*(3), 1756-1766.

[2] Herrero Acero, E., Ribitsch, D., Steinkellner, G., Gruber, K., Greimel, K., Eiteljoerg, I., ... & Guebitz, G. (2011). Enzymatic surface hydrolysis of PET: effect of structural diversity on kinetic properties of cutinases from Thermobifida. *Macromolecules*, *44*(12), 4632-4640.

[3] Pellis, A., Ferrario, V., Cespugli, M., Corici, L., Guarneri, A., Zartl, B., ... & Gardossi, L. (2017). Fully renewable polyesters via polycondensation catalyzed by Thermobifida cellulosilytica cutinase 1: an integrated approach. *Green Chemistry*, *19*(2), 490-502.

[4] Weinberger, S., Pellis, A., Comerford, J. W., Farmer, T. J., & Guebitz, G. M. (2018). Efficient physisorption of Candida Antarctica Lipase B on Polypropylene Beads and application for polyester synthesis. *Catalysts*, *8*(9), 369.

3. Lidija Kenjeric

FFoQSI GmbH, FFoQSI Austrian Competence Centre for Feed & Food Quality, Safety and Innovation, Tulln, Austria

Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Konrad-Lorenz-Straße 20, 3430 Tulln an der Donau, Austria

Lidija.Kenjeric@ffoqsi.at

Development and validation of an HPLC-MS/MS multi-class method for the analysis of different classes of veterinary drug residues in milk and poultry feed

Lidija Kenjeric [1], Michael Sulyok[2], Alexandra Malachova[1], David Steiner[4], Rudolf Krska[2,3], Brian Quinn[3], Brett Greer[3], Christopher T. Elliott[3, 5]

[1] FFoQSI GmbH, FFoQSI Austrian Competence Centre for Feed & Food Quality, Safety, and Innovation, Tulln, Austria
[2] University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology, Institute of Environmental Biotechnology, Konrad Lorenz Strasse 20, 3430, Tulln an der Donau, Austria
[3] Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, University Road, Belfast, BT7 1NN, Northern Ireland, United Kingdom
[4] Romer Labs Diagnostic GmbH, Analytical Service Department, Technopark 5, 3430 Tulln
[5] School of Food Science and Technology, Faculty of Science and Technology, Thammasat University, 99 Mhu 18, Pahonyothin Road, Khong Luang, Pathum Thani 12120, Thailand

Constant use of veterinary drugs in livestock leads to issues such as residue monitoring, cumulative risk assessment, antimicrobial resistance, and environmental contamination. As a consequence, it is important to set regulatory limits and maintain them with the help of reliable analytical methods. However, the development of a comprehensive analytical method for different compound classes that saves time but retains sensitivity and robustness is still an ongoing task globally. This study aimed to develop and validate an HPLC-MS/MS multi-class method for the analysis of different types of veterinary drug residues in milk and poultry feed. The sample preparation protocol based on the "dilute and shoot" approach previously used for multi-mycotoxin detection (Malachová et al. (2014)) was followed and further optimized in this study. Method validation for >150 analytes was conducted according to the SANTE validation guideline. Method performance characteristics such as linearity, limits of detection, limits of quantification, precision, accuracy, and repeatability were examined. Achieved limits of detection were lower than maximal residual limits (MRLs) for veterinary drug residues in milk for the vast majority of analytes. Limits of quantification for >80% of the analytes in milk were between 10 and 50 µg/kg and lower, while for poultry feed regulations of MRL limits are still not available. Intermediate precision complied with the SANTE criterion of RSD <20% for almost 90% of the analytes. Milk samples were affected by matrix effects with a signal enhancement for 25% of analytes above 120% compared to solvent standards. In contrast, strong signal suppression was observed in poultry feed, a much more complex matrix, with 40% of analytes detected below 70% when compared to the solvent standards. The validation results show that a majority of analytes (80-90%) comply with the SANTE criteria for accuracy with a recovery of the extraction of 70-120%.

Acknowledgements

The COMET-K1 competence center FFoQSI is funded by the Austrian federal ministries BMK, BMDW, and the Austrian provinces of Lower Austria, Upper Austria, and Vienna within the scope of COMET - Competence Centers for Excellent Technologies. The program COMET is handled by the Austrian Research Promotion Agency FFG.

4. Tobias Nenning

University of Natural Resources and Life Sciences, Vienna, Department of Material Sciences and Process Engineering, Institute of Wood Technology and Renewable Materials, Konrad-Lorenz-Straße 24, 343, Tulln an der Donau, Austria

tobias.nenning@boku.ac.at

From branch to beam – towards a resource efficient material concept for low value hardwood assortments

Tobias Nenning[1], Johannes Konnerth[1], Michael Grabner[1], Christian Hansmann[2], Wolfgang Gindl-Altmutter[1] and Maximilian Pramreiter[1]

[1] University of Natural Resources and Life Sciences, Vienna Department of Material Sciences and Process Engineering Institute of Wood Technology and Renewable Materials
[2] Wood K plus – Competence Centre for Wood Composites and Wood Chemistry

Climate change adaptation requires a shift in vegetation away from softwood forests to potentially more resilient hardwood and mixed forest stands [1]. A trend that is already evident in Austria's forests: Coniferous stands have decreased by 6% in the last decade, and mixed deciduous forests and pure deciduous forests have increased by 6% and 8%, respectively [2]. Since hardwoods differ fundamentally in their habitus and mechanical-technological properties from the softwoods currently dominant in Europe, new approaches to boost hardwood utilization are urgently needed. Therefore, my research is addressing a new pathway to producing high-value wood products for structural building components from low-value non-sawmill grade hardwood tree branches and stem tops.

The route towards this aim consists of the following steps: firstly, fundamental characterization of physical-, and mechanical properties of wooden branches from *Fagus sylvatica, Quercus petraea* and *Populus alba*. Subsequently, mapping of material properties into a digital-tree including its branches by means of personal laser scanning. Secondly, splitting of branches along the grain to produce elongated macro-strands with minimised fibre deviation and thirdly, straightening with the aid of thermally assisted plasticisation in steam and densification to form straight fibre elements capable of serving as structural beam elements e.g. in construction.

This highly material-efficient utilisation concept for low-value hardwood is expected to enable a high proportion of bio-based material utilisation also in future.

References

[1] Schier F, Morland C, Janzen N, Weimar H (2018) Impacts of changing coniferous and non-coniferous wood supply on forest product markets: a German scenario case study. European Journal of Forest Research 137 (3):279-300. doi:10.1007/s10342-018-1111-6
[2] Bundesforschungszentrum für Wald (2022) Österreichische Waldinventur. Retrieved from: www.waldinventur.at [18.08.2022]

Acknowledgements

We gratefully acknowledge financial support from NÖ Forschungs- und Bildungsges.m.b.H. (NFB), Neue Herrengasse 10, 3. Stock, 3100 St. Pölten, Österreich. FTI20-003

5. Felix Neudecker

Institute of Wood Technology and Renewable Materials,
University of Natural Resources and Life sciences, UFT-Konrad Lorenz-Straße 24, Tulln an der Donau

Felix.neudecker@boku.ac.at

Upgrading cereal straw to versatile high-value materials

Neudecker Felix[1], Gindl-Altmutter Wolfgang[1], Veigel Stefan[1]

[1] Institute of Wood Technology and Renewable Materials, Department of Material Sciences and Process Engineering, University of Natural Resources and Life sciences (BOKU)

In order to transform our currently fossil-based economy into a modern bio-based economy, new utilisation concepts for the available biomaterials are required. Here, the material use of underutilized agricultural by-products like cereal straw offers considerable potential. Due to its high cellulose content, cereal straw shows excellent strength properties, thus representing a promising resource for the production of structural engineering materials.
Our research project aims at utilising the naturally optimised and complex structure of straw to produce a mechanically powerful bio-based material. Therefore, the fundamental approach of delignification followed by densification was chosen to maximise the mechanical properties of straw and assemble it into a structural material.
In the first project step, we developed a manufacturing process by which test specimens with mechanical performance similar to established wood-based construction materials could be prepared. By lignin removal, the process first generates well malleable straw elements with increased relative cellulose content which can then be processed into a material combining high density, homogeneous structure and optimized fibre orientation. Building on these promising findings, my research aims to develop high-value structural materials based on cereal straw for real-life applications.

Acknowledgements
The authors are thankful for financial support for the project "Upgrading agro-residues to versatile high value materials" funded by NÖ Forschungs- und Bildungsges.m.b.H. (NFB), Neue Herrengasse 10, 3. Stock, 3100 St. Pölten, Austria.

6. Billich Elisabeth

Institute of Chemistry of Renewable Resources, Department of Chemistry, BOKU University of Natural Resources and Life Sciences, Gregor-Mendel-Stralße 33, 1180 Wien

lisa.billich@students.boku.ac.at

Biobased Impregnation Resins for the core layer of high-pressure laminates

Billich Elisabeth[1,2], Hogger Elfriede[2], van Herwijnen Hendrikus W.G. [2]

[1] Institute of Chemistry of Renewable Resources, Department of Chemistry, BOKU University of Natural Resources and Life Sciences
[2] Kompetenzzentrum Holz GmbH Wood K plus, 4040 Linz, Austria

High pressure laminates (HPL) are engineered materials that are used as both interior and exterior decorative horizontal and vertical surfaces that require demanding properties in terms of high wear, abrasion and stain resistance. They can be employed for a wide range of applications, including the construction of furniture, countertops, flooring, as well as wall cladding, facades and balconies. HPL consist of various layers comprising a core layer of several sheets of Kraft paper, a top layer of decorative paper and an optional wear layer. The respective paper in the layers is impregnated with low viscous resins. For the core layer, phenol-formaldehyde (PF) resins are commonly used for impregnation. This process renders the final product resistant to chemicals, moisture and heat, and imparts excellent mechanical properties. However, the two main constituents of PF resins are derived from fossil raw materials. Environmental and climate protection are becoming increasingly important. Many industries, including the laminate industry, are therefore striving to integrate more sustainable resources into their production processes. With these considerations in mind, the aim of this project is to find an alternative to conventional PF resins. A bio-based impregnation resin is to be developed, consisting mainly of renewable raw materials and non-hazardous chemicals, aiming at protecting the environment. To date, several approaches for modified PF resins, in which a portion of phenol was replaced by natural phenolic resources, have been reported in the literature, but a genuine alternative has not yet been found.

Acknowledgements
The authors gratefully acknowledge the financial support of the Austrian Research Promotion Agency (FFG) (project Nr: F0999891639). This project is funded by the Forest Fund, an initiative of the Federal Ministry of Agriculture, Forestry, Regions and Water Management and is carried out as part of the Think.Wood program of the Austrian Wood Initiative.

7. Sidhant Satya Prakash Padhi

Institute of Environmental Biotechnology, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
Kompetenzzentrum Holz GmbH (Wood K plus), Linz, Austria

sidhant.padhi@boku.ac.at/s.padhi@wood-kplus.at

Synthesis of three-component bio-based thermosets using oxidized starch, lignin and amines

Sidhant Satya Prakash Padhi[1,2], Alessandro Pellis[2,4], Hendrikus W. G. van Herwijnen[1,3], Georg M. Guebitz[2]

[1] Kompetenzzentrum Holz GmbH (Wood K plus), Linz, Austria
[2] Institute of Environmental Biotechnology, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
[3] Institute of Wood Technology and Renewable Materials, Department of Material Sciences and Process Engineering, University of Natural Resources and Life Sciences, Vienna, Austria
[4] Dipartimento di Chimica e Chimica Industriale, Università degli Studi di Genova, Genova, Italy

Adhesives used in the manufacture of the wood composites have a contributing influence on the properties of the final product. Even though conventional adhesives use formaldehyde as an effective crosslinker, its classification as a carcinogenic compound creates an urge to develop greener and more sustainable alternatives. In this work, the bio-based adhesives were prepared by polycondensation reaction of oxidized starch with hexamethylenediamine and lignin by varying molar ratios and reaction conditions. The morphology, stability, molecular structure, bond strength, viscosity and curing temperatures of the adhesive were investigated. The influence factors like type of starch, solid content and the effect of amine on bonding strength were considered and the results showed that an oxidized starch and lignin system reached a strength of 5.5±0.5 MPa while addition of amine is beneficial to the system with bond strengths reaching up to 10±1 MPa and 100% wood failure. Moreover, it was also found out that the three-component system remained stable at a solid content of 36% with a viscosity of 170 mPa.s at a shear rate of 100/s. Thermal analysis (DSC and TGA) were also used to measure curing and decomposition temperature of the system, which showed an onset of curing at 98°C and a decomposition at 280°C. In conclusion, the three-component thermoset here developed can be considered as a greener alternative to the conventional systems.

Acknowledgements
This work was supported by the government of Lower Austria under the project name “BioSET”. The consortium consists of research institutes like Institute of Environmental Biotechnology, IFA-Tulln, BOKU; Institute of Applied Synthetic Chemistry, TU Wien; Area 4 – Wood material technologies, Kompetenzzentrum Holz GmbH and industry partners such as Agrana Research and Innovation Centre GmbH; Metadynea GmbH; Murexin GmbH and Saapi GmbH.

8.Jonathan Samson

Institute for Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Konrad Lorenz Str. 20, 3430 Tulln, Austria

jonathan.samson@boku.ac.at

MetInclude: A Method To Streamline Untargeted LC-HRMS/MS Measurements Through Optimized, User-Configurable Inclusion Lists.
A User-Configurable Method for Constructing Optimized Inclusion Lists for Untargeted LC-HRMS/MS Measurements

Jonathan Samson[1], Christoph Bueschl[1], Maria Doppler[1], Kristina Missbach[1], Bernhard Seidl[1], Rainer Schuhmacher[1],

[1] BOKU-University of Natural Resources and Life Sciences, IFA Tulln, Austria

Untargeted metabolomics is often performed using chromatography paired with a high resolution tandem mass spectrometer (HRMS/MS), separating metabolites based on retention time and mass to charge ratio (M/Z). When using liquid chromatography (LC), the retention time isn't enough to identify the metabolites, so a fragmentation step followed by separation of the fragments by M/Z are used. The information gathered by the fragmentation pattern can help the annotation or identification of the metabolite.

Data-dependant acquisition (DDA) methods select LC-HRMS/MS precursor ions for fragmentation based on their intensity values. The highest intensity precursors are selected first. Thus, mass peaks that represent high background signals are often chosen in lieu of true biochemical sample constituents. This can critically limit the number of fragmented, biologically relevant compounds.

We present MetInclude, a data-set dependant acquisition (DsDA) method that utilizes the open source tool XCMS to generate globally optimized inclusion lists from LC-HRMS chromatograms. MetInclude automates background subtraction and only includes the monoisotopic peak of features that have 13 C isotopologs. The detected features are then split into separate inclusion lists based on relative signal intensity. Samples that have more coeluting metabolites than are allowed to be fragmented after a sin-gle full scan will cause additional inclusion lists to be created. The inclusion lists generated by MetInclude can be imported directly to support LC-HRMS/MS method setup for MassHunter- and XCalibur-based instruments.MetInclude has shown >93% coverage of truly biologically-derived features. As MetInclude uses a peak-picking algorithm, it is an improvement to DDA-based precursor ion selection, and can achieve global coverage of informative fragmentation spectra while reducing the number of samples and measurements needed. MetInclude is able to minimize false-positives compared to DDA, increasing the proportion of informative MS/MS spectra.

Acknowledgements
The authors would like to thank the Provincial Government of Lower Austria (OMICS 4.0 project) for funding this work.

9. Christina Maisl

Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology IFA-Tulln, University of Natural Resources and Life Sciences (Vienna), Konrad-Lorenz-Str. 20, 3430 Tulln, Austria

christina.maisl@boku.ac.at

Untargeted plant metabolomics: Evaluation of lyophilization as a sample preparation technique

Christina Maisl[1], Maria Doppler[1], Bernhard Seidl[1], Christoph Bueschl[1], Rainer Schuhmacher[1]

[1] Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology IFA-Tulln, University of Natural Resources and Life Sciences, Vienna

Lyophilization is a technique frequently used for stabilizing and concentrating biological samples or extracts prior to storage and further sample preparation. As with each sample preparation step, it is possible that metabolites and their concentrations are unintentionally altered or sample constituents may even be completely lost. However, the extent of this potential problem is not known so far. Here, the loss and alteration of metabolites during lyophilization prior to LC-HRMS analysis is investigated at the example of plant roots.

To investigate the performance of extract concentration by lyophilization, both an untargeted approach employing native and ^{13}C-labeled wheat roots and a targeted approach monitoring a mix of 16 reference standards were used. After extraction with water, different dilution levels (1-32x) were established to simulate samples like, e.g. root exudates, which are poor in constituents. Next, the diluted extracts were lyophilized to dryness, reconstituted in HPLC injection solvent and their metabolic composition compared to the original (non-lyophilized) extract.

Additionally, the effect of lyophilization prior to sample extraction was investigated. Fresh and lyophilized plant material was extracted with methanol/acetonitrile/water 3:3:2 (v/v/v) and their metabolic compositions were compared.

All extracts were analyzed using RP-LC-HRMS and data were processed with the in-house developed software tool MetExtract II.

The applied, well-established isotope-assisted workflow enabled to detect several hundred metabolites and to efficiently filter out all non-plant derived background ions. Relative abundances of samples with and without lyophilization were highly reproducible (median RSD about 10%). Of all metabolites present in the original extracts, less than 5% were not detected in the lyophilized extracts. Moreover, lyophilization successfully enriched the diluted extracts for the majority (over 80%) of metabolites. On the contrary, the study demonstrated qualitative and quantitative differences between fresh and lyophilized root samples (i.e. extraction after lyophilization). About 50% of the totally detected metabolites were affected quantitatively.

Acknowledgements

We gratefully acknowledge financial support from the Austrian Science Fund (FWF), playNICE project (ZK-74).