Construction of Functional Bacterial Chassis and Synthetic Consortia to Produce Bio-Based Platform Chemicals from Lignin-Derived Aromatics

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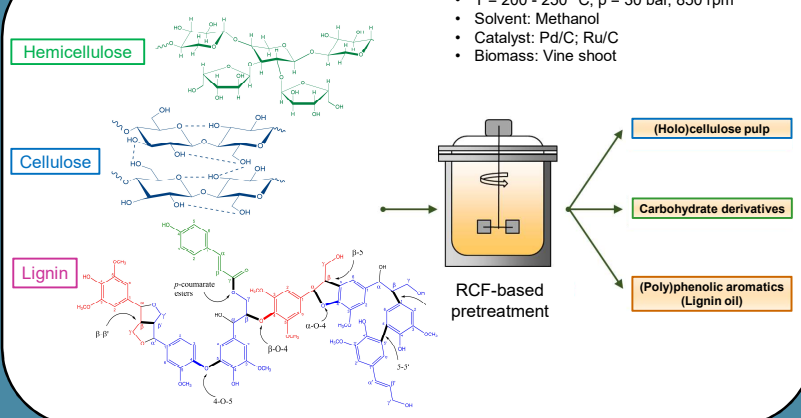
Today, around 40% of the global industrial chemical production is based on aromatics, which are primarily made from limited petrochemical resources. As the largest renewable source of aromatic compounds, lignin fraction from lignocellulosic biomass represents a promising alternative to reduce this dependency. In this context, the research project seeks to valorize lignin by combining reductive catalytic fractionation (RCF) as biomass pretreatment with microbial funneling of the resulting lignin-derived monoaromatics into platform molecules and value-added products.

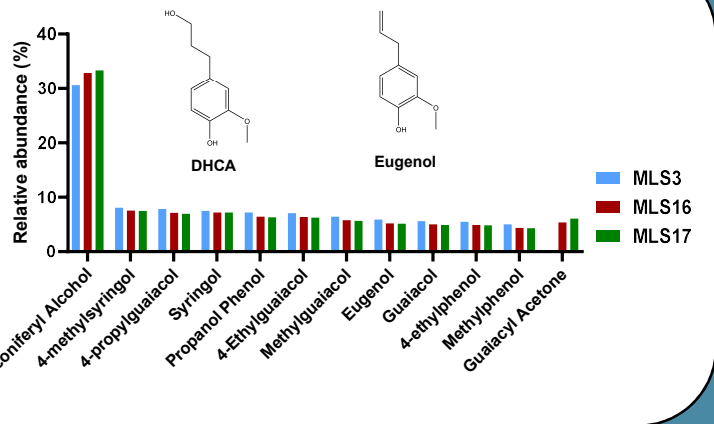
Reductive catalytic fractionation

Pretreatment conditions [1]

- Reducing agent: H_2
- T = 200 - 250 °C, p = 30 bar, 850 rpm
- Solvent: Methanol
- Catalyst: Pd/C; Ru/C
- Biomass: Vine shoot



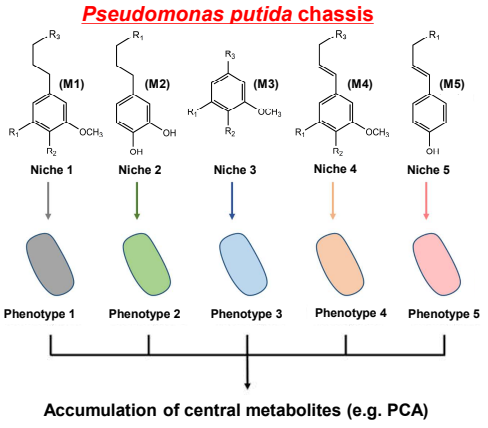
Aromatic composition of lignin oil extracted from vine shoot [2]



Current industrial biorefining processes mainly focus on the recovery of holocellulose and produce low quality, degraded lignin fractions that are mostly only worth converting into thermal energy. Therefore, a RCF process is being developed as a lignin-first approach to maintain as much as possible the native functionalities of lignin upon fractionation. Native-like lignin streams are then converted into functionalized bio-based aromatics. The proposed project seeks to create a more integrated and sustainable biorefinery, by maximizing the utilization of the entire lignocellulosic biomass with full economic efficiency.

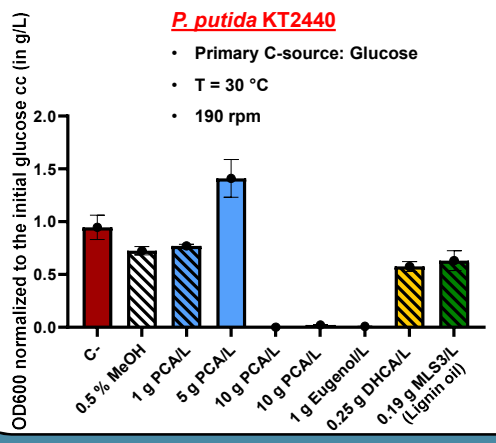
Biological funneling

***Pseudomonas putida* chassis**



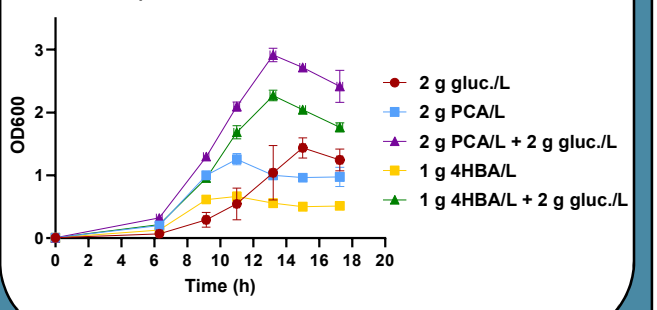
***P. putida* KT2440**

- Primary C-source: Glucose
- T = 30 °C
- 190 rpm



***P. putida* SEM10**

- C-source: Glucose, PCA or 4-hydroxybenzoic acid (4HBA)
- T = 30 °C
- 190 rpm

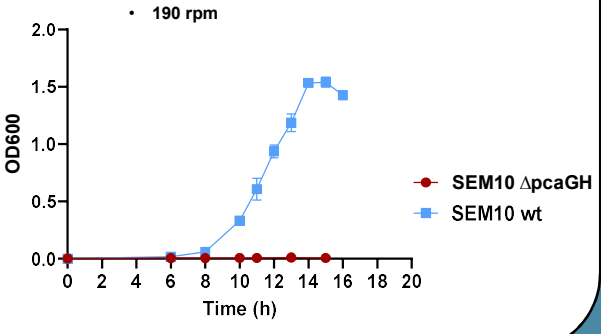


Pseudomonas putida strains KT2440 and SEM10[3] were investigated as bacterial chassis for the conversion of the heterogeneous mixture of lignin-derived monoaromatics obtained from RCF into platform chemicals such as protocatechuate (PCA). SEM10 is a genome-streamlined strain derived from *P. putida* KT2440 and shows superior growth characteristics compared to its parental strain. Both strains have shown ability to tolerate various aromatics of interest. SEM10 will be used to develop custom-made functional bacterial chassis for metabolic funneling into central cellular metabolism and, ultimately, biosynthesis of target compounds.

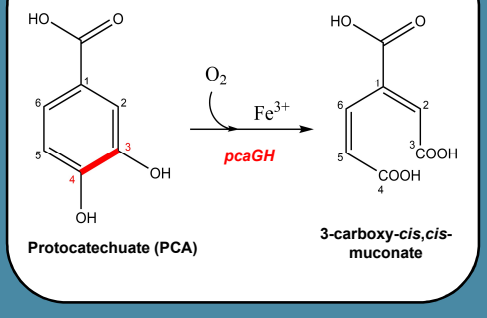
Screening for genes of interest

***P. putida* SEM10 ΔpcaGH**

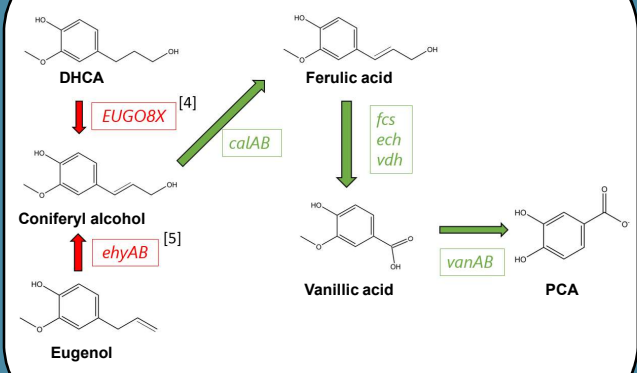
- 2 g PCA/L
- T = 30 °C
- 190 rpm



***pcaGH* deletion**



***EUGO8X* and *ehyAB* insertion**



By following the protocol from Volke et al. (2021)[6], strain SEM10 *ΔpcaGH* was successfully engineered as a first trial for PCA accumulation. The research now primarily focuses on the insertion of genes into the SEM10 chassis. Genes involved in the metabolism of DHCA and eugenol have been identified in *Rhodococcus jostii* RHA1 and *P. putida* HR199, respectively, and will be tested for biological funneling and PCA accumulation.

[1] Brienza et al., 2021; Nishimura et al., 2018
[2] Adapted from D. Debecker and M. L. Serrano's unpublished research
[3] Volke et al., 2020
[4] Guo et al., 2023
[5] Brandt et al., 2001
[6] Volke et al., 2021

Funding: FSR grant 2023, UCLouvain

In collaboration with P. Nikel and D. Volke, The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark