Pseudomonas putida's tolerance to toxic biorefinery waste streams has been improved by 200-fold

Background

- **Thermochemical conversion of lignocellulose** (i.e., catalytic fast pyrolysis) for the production of fuels and chemicals generate toxic wastewater streams that require costly waste treatment processes.
- Pyrolysis streams contain, among other molecules, **glycolaldehyde, acetate, and phenolic compounds** (up to 350 g/L carbon content), which exhibit severe microbial toxicity.
- This study focuses on **increasing the tolerance of *P. putida*** to these streams and demonstrating production of polyhydroxyalkanoates (PHA), a value-added compound.

Approach

- Inhibition of *P. putida* **growth rates** was evaluated in context to pyrolysis chemicals to identify the main source of toxicity.
- A **multi-omic analysis** was performed to identify gene targets of *P. putida* in response to pyrolysis streams and glycolaldehyde.
- After selecting several targets, *P. putida* was **genetically modified** to evaluate if the tolerance was improved.
- Pyrolysis streams were utilized as only carbon source in *P. putida* cultivations to demonstrate thier conversion to PHA.

Outcomes

- Aldehydes are the major contributor to the toxicity of pyrolysis streams.
- The overexpression of the **chaperone genes -- *clpB*, *groES*, and *groEL*** in a genome-reduced *P. putida* strain enhanced the tolerance to a variety of pyrolysis streams (up to 200-fold) compared to the native *P. putida* KT2440. These genetic combinations lead to a more efficient protein folding, higher ATP production, and greater abundance of cellular defense proteins.
- *P. putida* was able to utilize a fraction of the waste stream as sole carbon and energy source and generate intracellular PHAs.

Significance

- The approach in this study can be utilized on different biorefinery streams as a **strain engineering strategy to overcome substrate or product toxicity.**
- The gene targets selected in this work may be key to enhance the tolerance of *P. putida* and other organisms to toxic **lignin streams**, a key substrate in CBI.

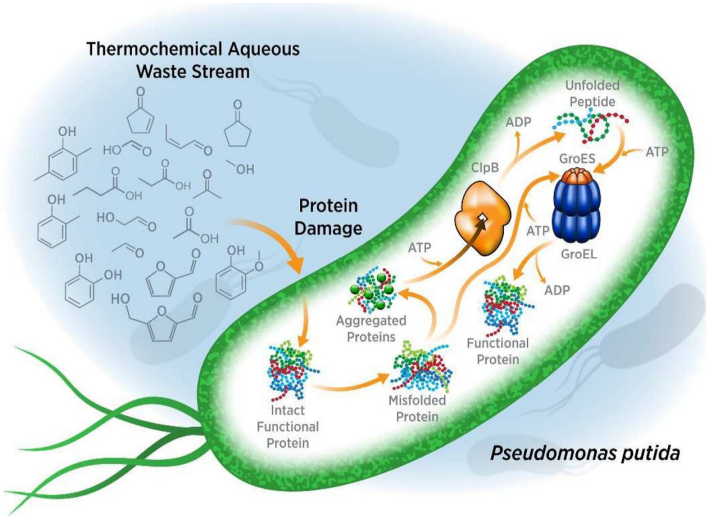

Illustration of the chaperone-dependent tolerance mechanism of engineered *P. putida* to thermochemical wastewater streams.

Jayakody, LN, Johnson CW., Whitman JM., Giannone RJ., Black, BA., Cleveland, NS., Klingeman, DM., Michener., WE., Olstad, JL., Vardon, DR., Brown RC., Brown SD., Hettich RL., Guss AM., Beckham GT. Thermochemical wastewater valorization via enhanced microbial toxicity tolerance. *Energy & Environmental Science*. 2018. DOI: 10.1039/C8EE00460A

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