

A new enzyme – a Rieske non-heme iron monooxygenases – allows rapid guaiacol O-demethylation for lignin catabolism

Background

- O-demethylation is a key step in microbial valorization of lignin-derived aromatic compounds such as guaiacol.
- In 2018, CBI identified the first known guaiacol demethylase, but its activity in a heterologous host is limited.
- Guaiacol is one of three primary lignin derived monomers

Approach

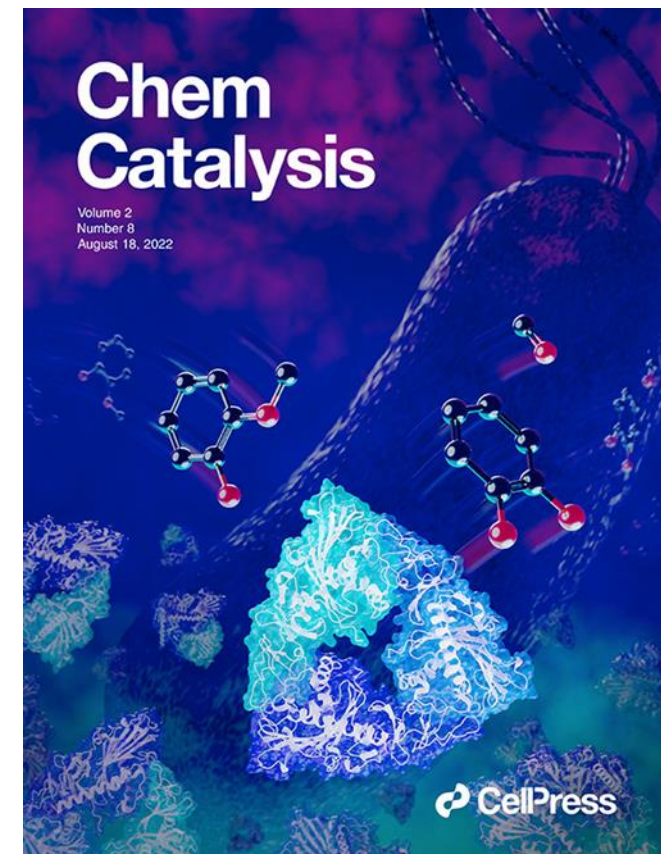
- An aromatic catabolic bacterium, *Novosphingobium aromaticivorans*, was evolved to rapidly catabolize guaiacol and randomly-barcoded transposon insertion sequencing was used to identify genes required for growth with guaiacol
- This screen identified a Rieske-type oxidative demethylase, now named GdmAB, that was purified, characterized, crystallized, and expressed in a heterologous host, *Pseudomonas putida*

Results

- The Rieske GdmAB guaiacol demethylase is approximately 40-fold more active in vitro than the previously-studied cytochrome P450 GcoAB guaiacol demethylase
- Analysis of the GdmAB crystal structure suggested key residues that determine substrate specificity compared to a related oxidative vanillate demethylase
- Heterologous expression of GdmAB in *P. putida* enabled rapid growth with guaiacol as the sole carbon source, reaching saturation in ~2 days compared to >10 days with the previous GcoAB guaiacol demethylase

Significance

- This study discovered a highly active enzyme for microbial valorization of a common lignin-derived compound with multiple applications in protein and metabolic engineering. This was confirmed by adding this aromatic catabolic capability into *P. putida* – a chassis microbe for lignin conversion.



A newly-discovered Rieske-type oxidative demethylase GdmAB catalyzes a key reaction in lignin valorization, the O-demethylation of guaiacol to catechol. (Journal Cover)

Bleem A., et al. *Chem Catalysis* (2022) <https://doi.org/10.1016/j.cheecat.2022.04.019>