

Lignin conversion to a performance-advantaged biochemical via *Pseudomonas putida*

Background

- The bioconversion of lignin-related aromatic compounds (LRCs) into valuable chemicals (i.e., β -keto adipic acid (β -KA), a building block for nylon-6,6 analogs) is needed for lignin valorization.
- *Pseudomonas putida* is a promising chassis for industrial bioconversion processes for complex substrates such as LRCs.
- *P. putida* KT2440 was previously engineered by disrupting *pcaIJ* (3-oxoadipate CoA transferase) for the conversion of LRCs into β -KA, but metabolic bottlenecks limit productivity and product titer.

Approach

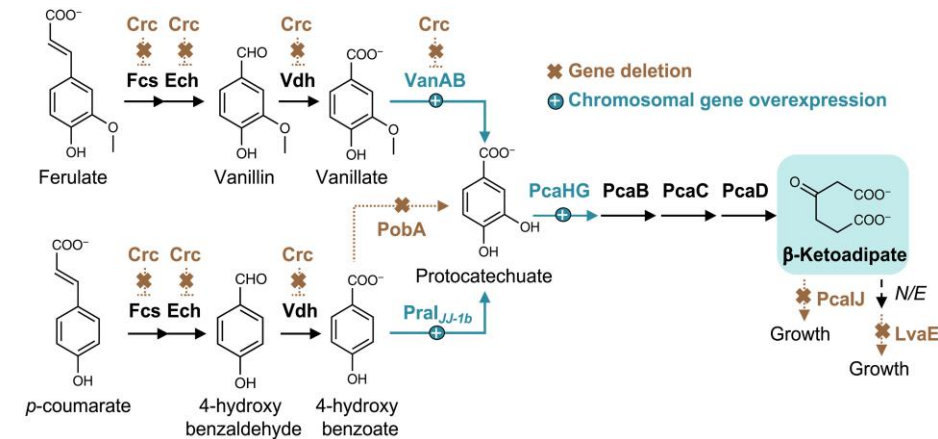
- We conducted iterative rounds of metabolic engineering and bioprocess development in *P. putida* KT2440, to convert LRCs to β -KA at high titer and productivity.
- We performed fed-batch bioreactor with constant feeding rates to maximize β -KA productivity.

Results

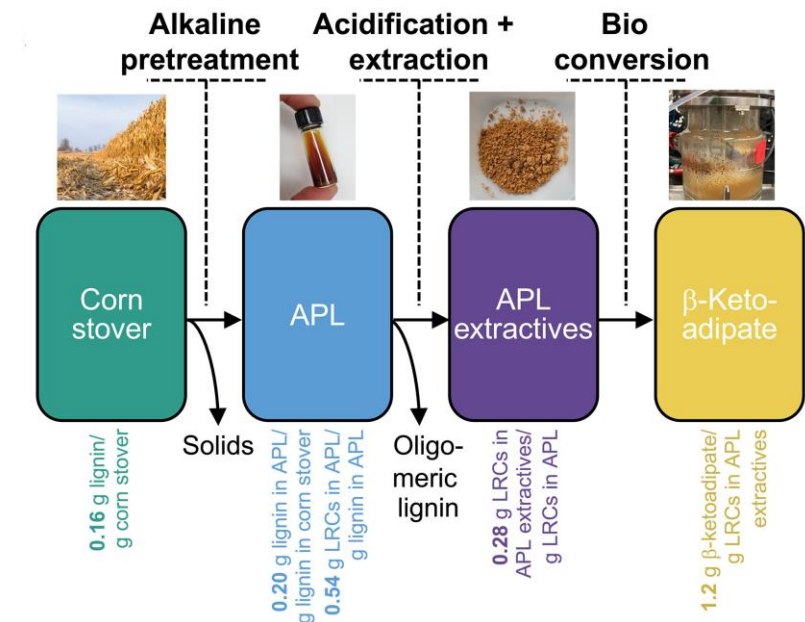
- We combined beneficial genetic interventions into a single production strain, *P. putida* AW299.
- AW299 produced 44.5 g/L of β -KA at 1.15 g/L/h from a 3:1 ratio mixture of *p*-coumarate and ferulate in a fed-batch bioreactor cultivation.
- Following extraction of *p*-coumarate and ferulate from corn stover alkaline pretreated liquor, AW299 produced 25 g/L of β -KA at a productivity of 0.66 g/L/h.
- Techno-economic analysis of the process predicted a β -KA minimum selling price of ~\$2/kg; this price is cost-competitive with fossil carbon-derived adipic acid (\$1.10-1.80/kg).

Significance

- This work demonstrates the highest product titer and productivity from biomass-derived aromatic compounds reported to date.
- This work highlights complete bioconversion of LRCs to higher value products can be cost-competitive with fossil carbon-derived products.



Metabolic pathway for the bioconversion of *p*-coumarate and ferulate to β -KA.



β -KA production from corn stover-derived lignin-related compounds

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