Adaptive evolution of *Pseudomonas putida* KT2440 improves aromatic catabolism and tolerance

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Background

- *P. putida* is a promising bacterial chassis for conversion of lignin-derived aromatic mixtures – enriched in *p*-coumaric (*p*CA) and ferulic (FA) acids – into valuable products.
- Improving microbial toxicity tolerance to aromatic compounds, such as *p*CA and FA, is necessary to achieve industrial relevance.
- Tolerance adaptive laboratory evolution (TALE) was employed to achieve this goal: *P. putida* was propagated into increasing concentrations of *p*CA, FA, or *p*CA+FA and the resulting evolved cells were whole genome sequenced (Fig. 1).

Conclusions

- Following TALE, lag phase in high *p*CA (20 g/L) was reduced 5.4-, 4.9-, and 4.4-fold in evolved strains TALE#7, #23, and #25 (Fig. 2a).
- Deletion of *PP_3350* – a genetic target identified via genome sequencing evolved isolates – in a wild-type background (CJ782) alone reduced lag phase in 20 g/L *p*CA by 4.1-fold (Fig. 2). Growth enhancements were also observed in a mixture of *p*CA and FA.
- Mutations in *TtgB,* part of the TtgABC resistance-nodulation-division efflux pump, contributed to growth improvements on FA and *p*CA.

Significance

- Improving strain performance for bacterial lignin valorization was achieved via TALE decreasing lag phase and increasing growth rate. Further improvements by this approach are possible.

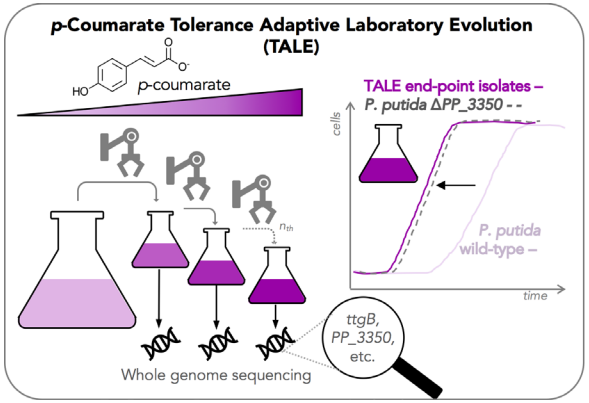

Fig. 1. Tolerance adaptive laboratory evolution experimental set-up.

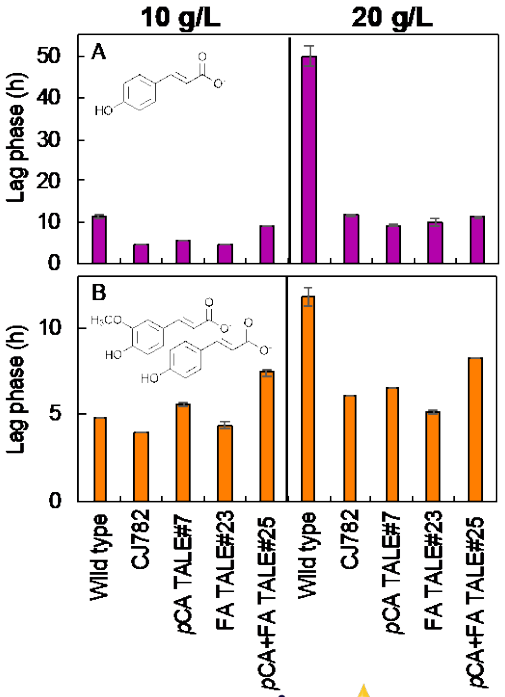

Fig. 2. Lag phase of wild-type *P. putida*, *P. putida* Δ*PP_3350* (CJ782), and TALE-evolved *P. putida* (#7, #23, and #25) in 10 or 20 g/L (**A**) *p*-CA or (**B**) *p*CA + FA provided at equal mass concentration. All TALE strains harbor *PP_3350* mutation(s).

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