Characterization of aromatic acid/proton symporters in *P. putida* KT2440 may allow more efficient microbial conversion of lignin-related aromatics

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Background

- *Pseudomonas putida* KT2440 is a well-studied platform bacterium for the production of industrially valuable chemicals. KT2440 can grow on lignin-related monomers, such as ferulate (FA), 4-coumarate (4CA), vanillate (VA), 4-hydroxybenzoate (4HBA), and protocatechuate (PCA). Genes associated with their catabolism are known, but knowledge about the uptake systems remains limited.

Approach

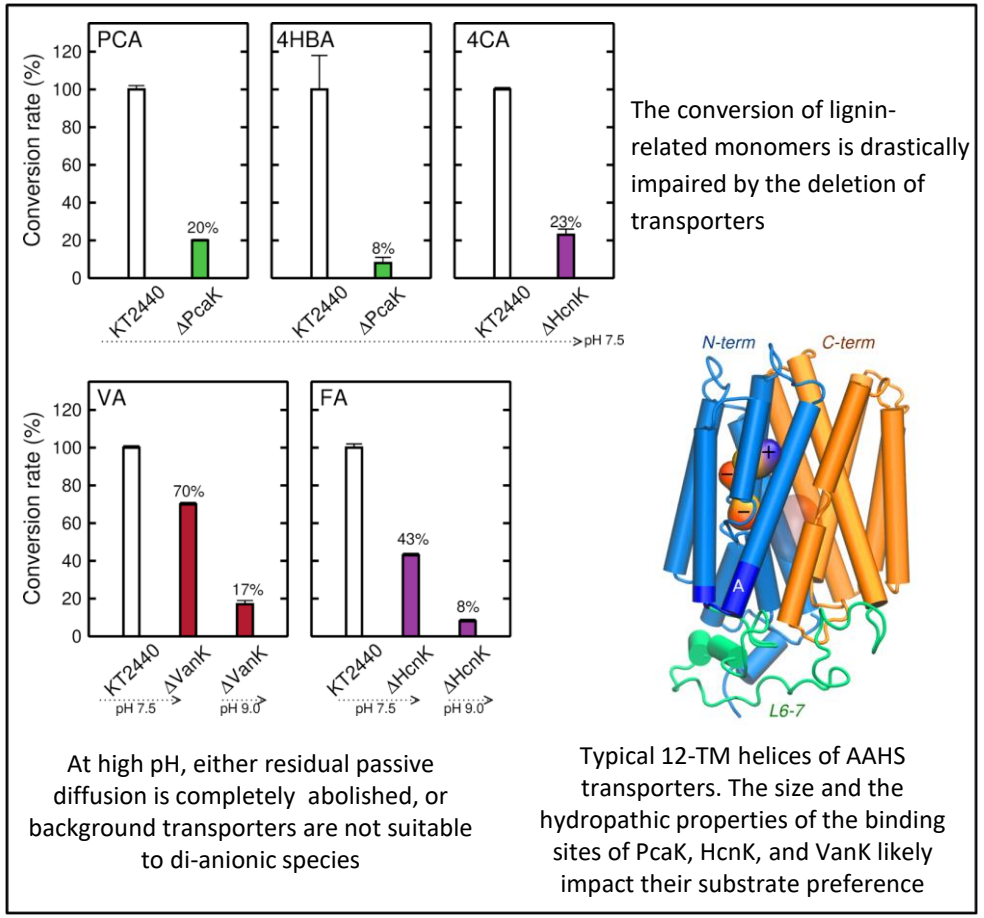
- We studied five KT2440 aromatic acid/H+ symporter family transporters of lignin-related monomers and their substrate selectivity.
- We performed uptake assays using C-labeled substrates, biosensor-based assays, and protein structure prediction/analysis.

Outcome

- Gene knockout experiments show that, in KT2440, PcaK, VanK, and HcnK are the major transporters of lignin-derived monomers.
- *Escherichia coli* cells expressing *pcaK*, *vanK*, and *hcnK* exhibit the capacities to uptake PCA/4HBA, VA/PCA, and FA/4CA, respectively.
- Comparative structural analysis suggests that the size and hydropathic properties of the substrate-binding sites of these transporters determine their substrate preferences.

Significance

- This study identified transporter genes are useful targets for enhanced microbial production of value-added compounds from lignin-derived aromatics and for better understanding of current limitations.



The conversion of lignin-related monomers is drastically impaired by the deletion of transporters

At high pH, either residual passive diffusion is completely abolished, or background transporters are not suitable to di-anionic species

Typical 12-TM helices of AAHS transporters. The size and the hydropathic properties of the binding sites of PcaK, HcnK, and VanK likely impact their substrate preference

A Wada, ET Prates, R Hirano, AZ Werner, N Kamimura, DA Jacobson, GT Beckham, E Masai, "Characterization of aromatic acid/proton symporters in *Pseudomonas putida* KT2440 toward efficient microbial conversion of lignin-related aromatics, Metabolic Engineering (2021), doi: https://doi.org/10.1016/j.ymben.2021.01.013.

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