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

**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.