Small Membrane Vesicles in *Pseudomonas putida* Harbor Aromatic-Catabolic Enzymes

Background/Objective

- In response to lignin, *P. putida* secretes a bimodal distribution of membrane vesicles (MVs) that conduct aromatic catabolism.
- Here we addressed two key questions: 1) which of the two MV populations (small or large) is responsible for extracellular aromatic turnover, and 2) how much of the aromatic turnover occurs extracellularly?

Approach

- We isolated the small and large MV during growth on lignin and control (glucose) substrates using asymmetric flow field-flow fractionation.
- We then characterized the aromatic-catabolic proteins in both the MVs and inside the cell using quantitative proteomics.
- Lipidomics of the MV fractions were also compared relative to the cell to understand if the membrane composition of MVs are tailored.

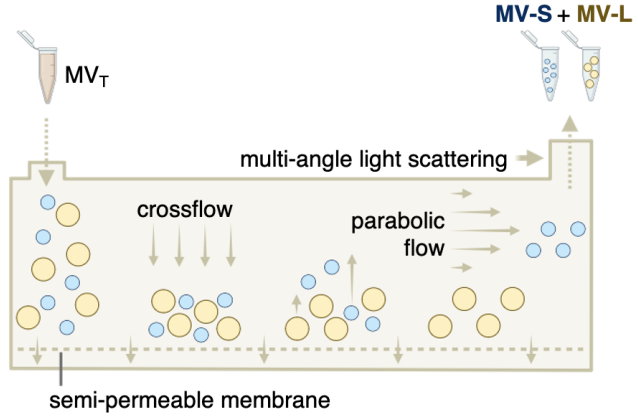
Results

- The small (diameter ~100 nm) and large (diameter ~300 nm) MV fractions have distinct protein cargo, with the small MV fraction harboring aromatic-catabolic enzymes and the large MV fraction included mostly outer membrane proteins.
- Relative to the intracellular protein fraction, the small MVs contained < 1% of the pool of any given β-ketoadipate pathway enzyme.
- MV lipids contained higher ratios of phosphatidylethanolamine:phosphatidylglycerol lipids, suggesting possible membrane customization for stability.

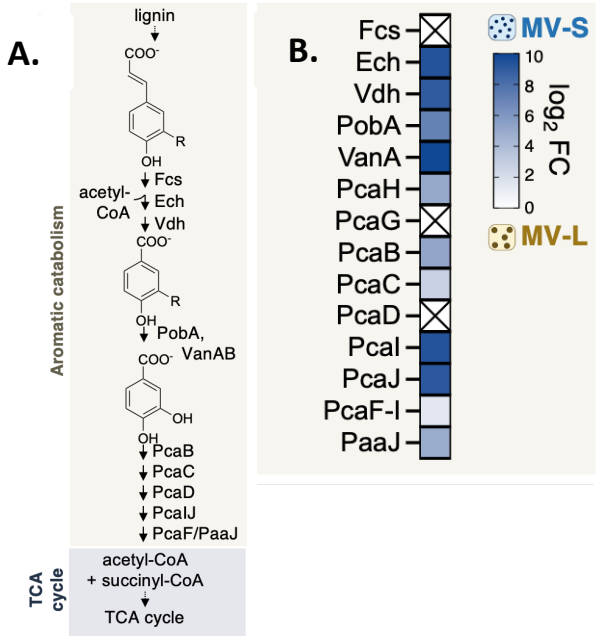
Significance/Impacts

- This work demonstrates that small MVs in *P. putida* are targeted for aromatic turnover in response to lignin substrates, and that, likely, the overall substrate turnover relative to the cell for aromatic monomers is considerably lower in MVs.

A.Z. Werner *et al., Appl. Environ. Microbiol.* 2025, /10.1128/aem.01617-25



Asymmetric flow field-flow fractionation to separate small (MV-S) and large (MV-L) MVs from cultivations of *P. putida* ± lignin substrates



A. β-ketoadipate (β-KA) pathway

B. Log$_2$ fold change for significantly enriched enzymes in the β-KA pathway in MV-S *vs.* MV-L