Engineered Membrane Vesicle Production via *oprF* or *oprI* Deletion Has Distinct Phenotypic Effects in *Pseudomonas putida*

Background/Objective

- Membrane vesicle (MV) production is a natural phenomenon in gram-negative bacteria, including *Pseudomonas putida* KT2440 (*P. putida*).
- Engineering MV production could enable improved production of biomolecules or bioproducts, but the impact of this on cellular phenotype in *P. putida* is unexplored.

Approach

- First, we constructed a library of *P. putida* genetic mutants targeting diverse functions (porins, lipoproteins, lipid shuffling/chaperones), and extracted and enumerated MV production in each.
- We identified hyper- and hypo-vesiculating mutants and characterized their effect on cellular fitness and the proteome, both within the cell and MVs, when grown on lignin-derived aromatic compounds.
- Finally, we engineered MVs as a mechanism for protein secretion and lipid production to improve both cellular fitness and MV secretion in hypervesiculation mutants.

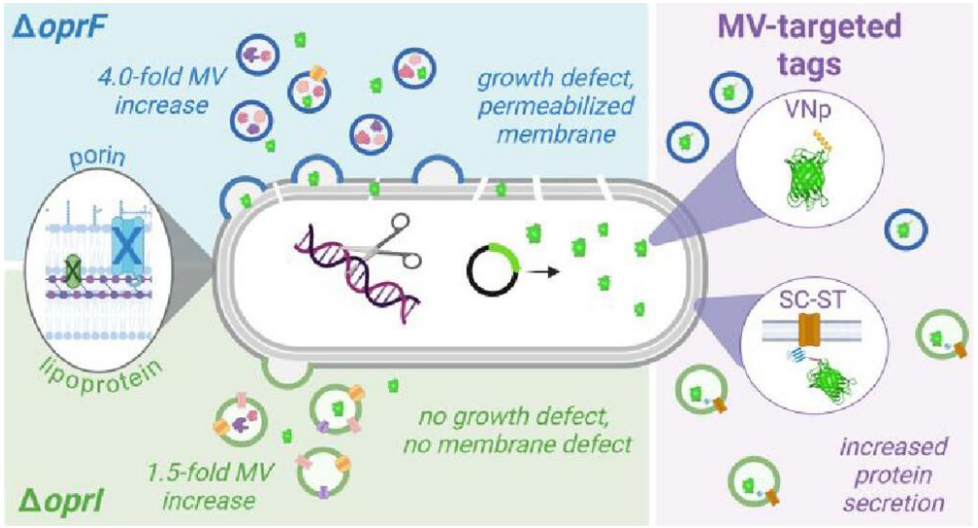

Fig. 1: Deletion of *oprF* and *oprI* both enabled membrane vesicle secretion, but with different impacts on cell fitness. Protein secretion was enabled via VNp and SC-ST tags, especially when combined with Δ*oprF* or Δ*oprI* mutations.

Results

- Deletion of *oprI,* encoding a lipoprotein, and deletion of *oprF*, encoding an outer membrane porin, both resulted in hypervesiculation but had distinct phenotypes, with Δ*oprF* having higher MV production but diminished growth likely due to a permeabilized membrane.
- Increasing glycerophospholipid biosynthesis via *gspA* overexpression improved membrane integrity without reducing MV production.
- Protein export was enabled via engineered SpyTag-SpyCatcher (SC-ST) and vesicle nucleating peptide (VNp) tags.

Significance/Impacts

- This study provides genetic engineering strategies with corresponding phenotypic outcomes that enable MV deployment as a synthetic biology tool in *P. putida,* a model CBI chassis for the production of biochemicals from lignin-derived aromatics.
- Engineering MVs could enable improved production of toxic compounds (e.g., aldehydes) via extracellular turnover.

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