Nutrient-dependent role of the outer membrane porin OprF in *Pseudomonas aeruginosa* biofilm formation

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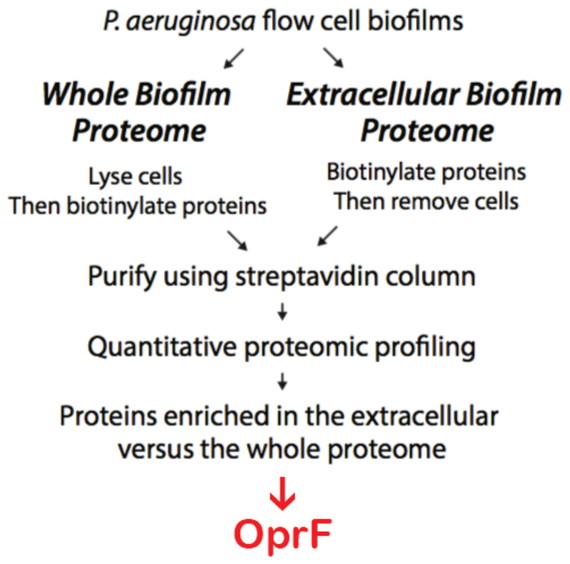
Why study biofilm matrix proteins?

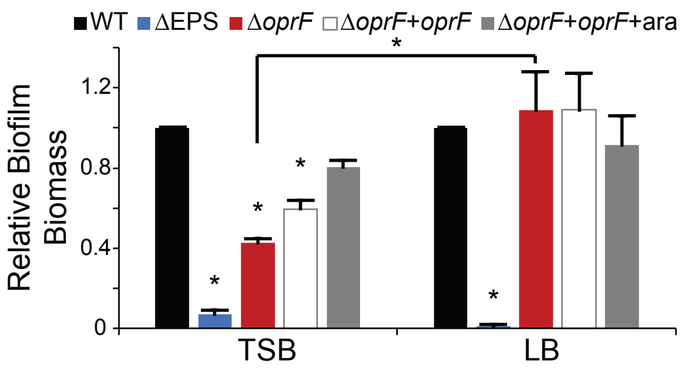
- Extracellular biofilm matrix protects resident cells from external stressors
- Relatively understudied matrix component
- Structural and biochemical functions within the matrix

OprF is a matrix protein



- Major non-specific outer membrane porin
- Homolog of *E. coli* OmpA
- Necessary for full virulence

OprF mutant biofilm formation



Biomass normalized to WT in same medium. Error bars, SEM (N=3); *, statistically different from WT in same medium ($p < 0.05$, ANOVA with post hoc Bonferroni).

The effect of OprF on biofilm formation is nutrient-dependent

Major media differences

- 5g/L NaCl
- 2.5g/L glucose
- 2.5g/L K_2HPO_4

- 10g/L NaCl

We hypothesize that differential TSB vs. LB Δ*oprF* biofilm formation is dependent on media components

Static biofilm assay in altered media

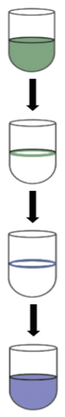
Original

- 5g/L NaCl
- w/ glucose
- w/ K_2HPO_4

Altered

10g/L NaCl | no glucose | no K_2HPO_4

All other components same



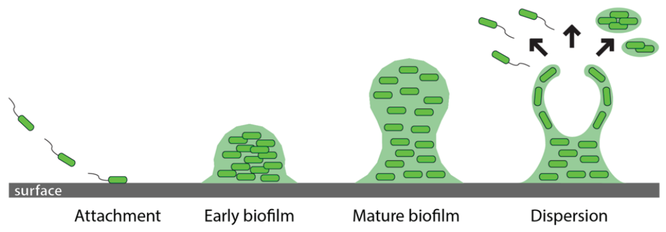
1) Inoculate strains into 96-well plate in medium of interest at 37˚C

2) Remove planktonic cells and wash wells with water

3) Stain attached biomass with crystal violet

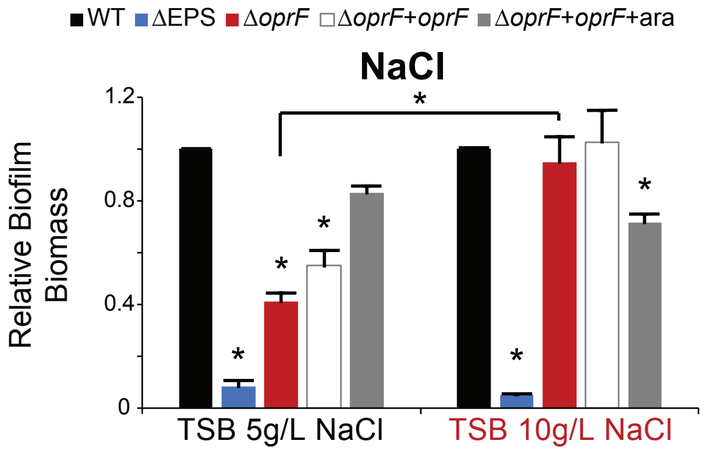
4) Solubilize stained biomass with acetic acid and read absorbance at OD_{550}

Stages of biofilm formation



Passos da Silva & Schofield et al., 2017

Altered media effects on Δ*oprF* biofilm

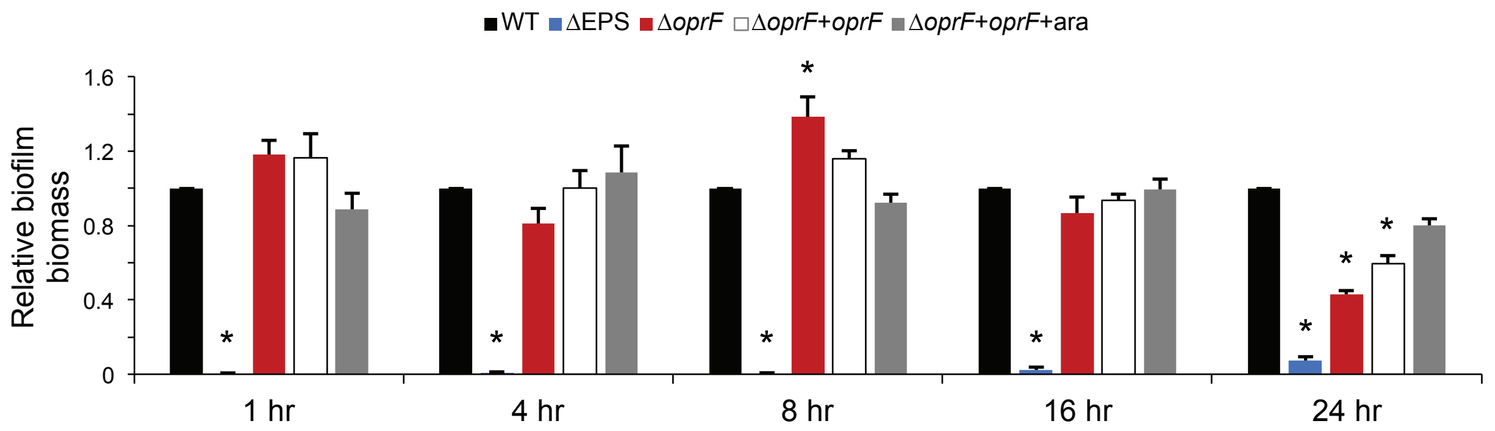


24-hr biomass normalized to WT in same medium. Error bars, SEM (N=3); *, statistically different from WT in same medium ($p < 0.05$, ANOVA with post hoc Bonferroni).

- K_2HPO_4 had no effect
- Reciprocal effects in LB

Nutrient-dependent effect of OprF on biofilm formation is due to NaCl and glucose concentrations

What are the temporal effects of OprF on biofilm formation?



Biomass normalized to WT at same time point. Error bars, SEM (N=3); *, statistically different from WT at same time point ($p < 0.05$, ANOVA with post hoc Bonferroni).

OprF affects late-stage static biofilm formation in TSB, between 16-24 hours

Future Directions

- Intracellular mechanisms behind nutrient-dependence: envelope stress response and c-di-GMP levels
- Extracellular effects: OprF mutant biofilm rescue by applying exogenous OprF

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