

Characterizing the role of denitrification in *Pseudomonas aeruginosa* biofilm formation

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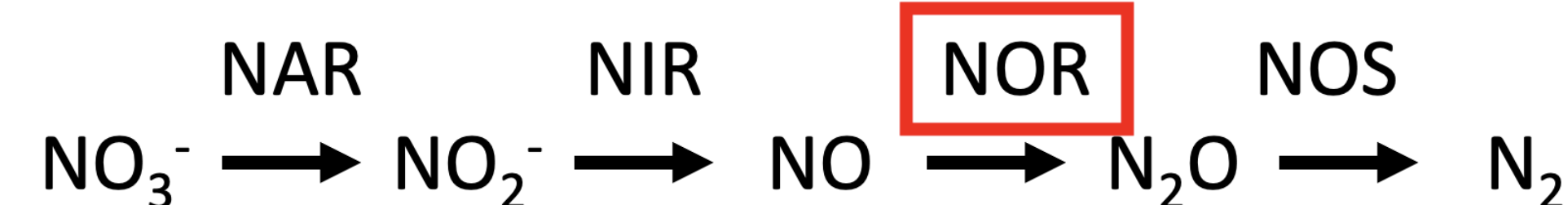
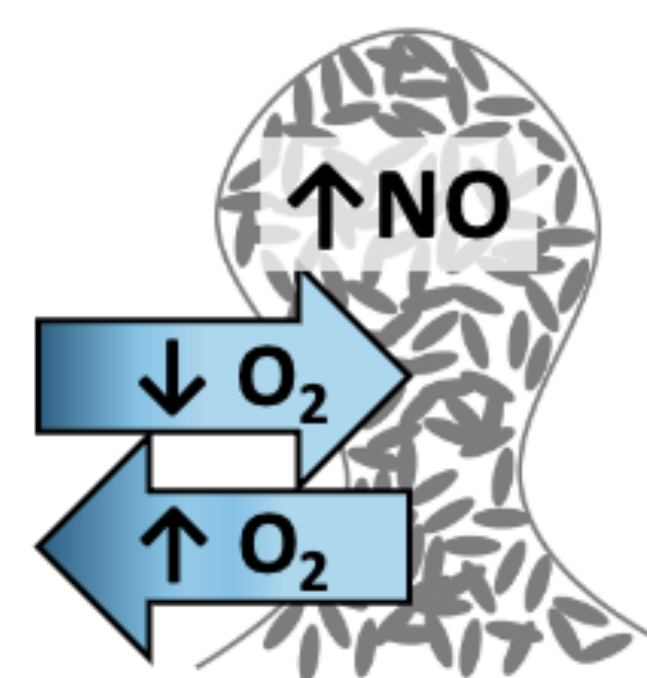


Why study biofilm formation?

- Biofilms are aggregates of bacteria that attach to surfaces and surround themselves in a gel-like protective matrix
- Biofilm-forming microbes, like *Pseudomonas aeruginosa*, are highly antibiotic and radiation tolerant
- Biofilms pose risk to astronaut safety, flight longevity, and forward planetary contamination

Impact of nitric oxide on microbes

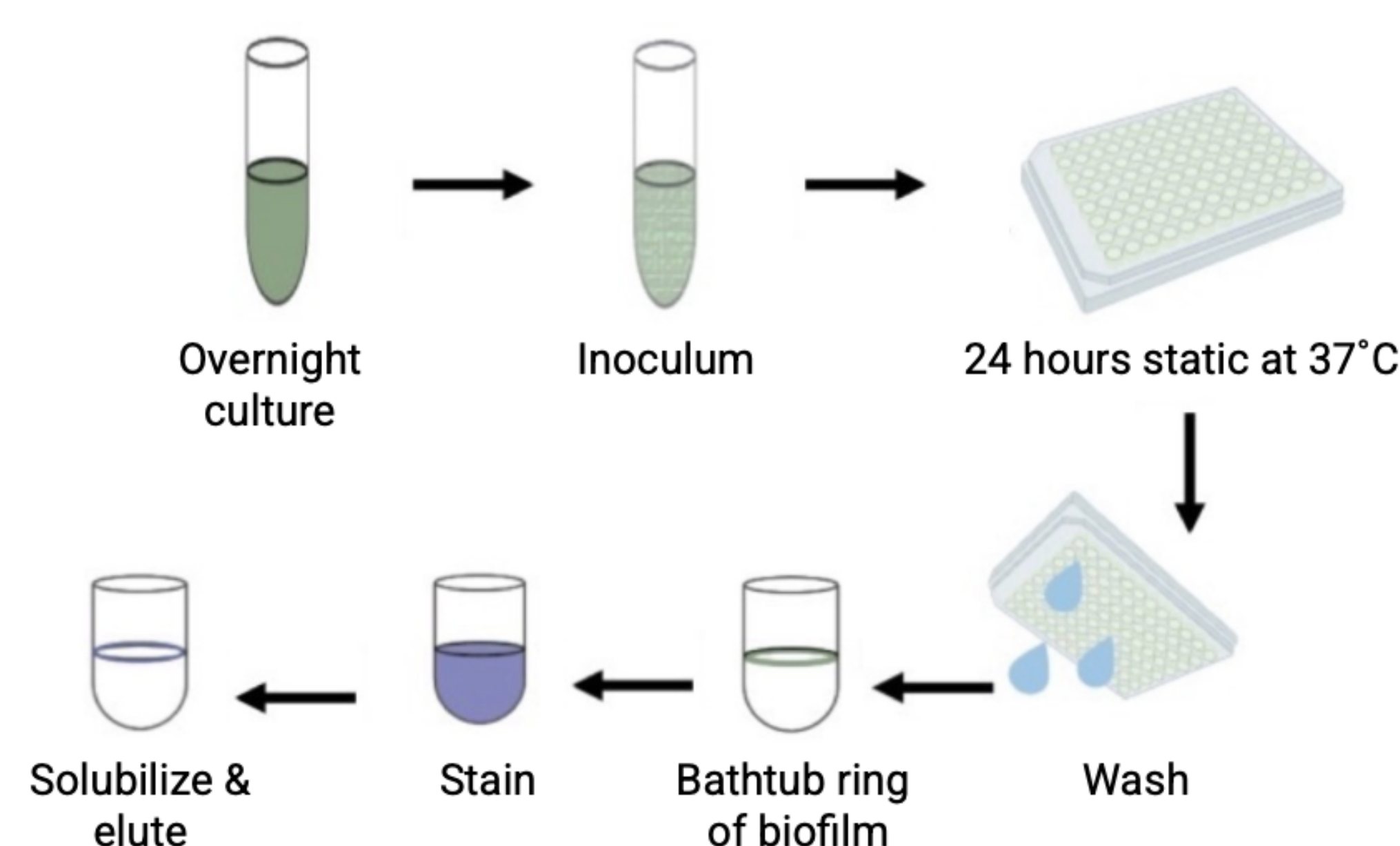
- Nitric oxide (NO) is a toxic reactive nitrogen species that accumulate in oxygen-depleted environments
- Nitric oxide reductase (NOR) reduces and detoxifies NO in the process of denitrification
- Shows promise as a method for controlling biofilm formation



Hypothesis

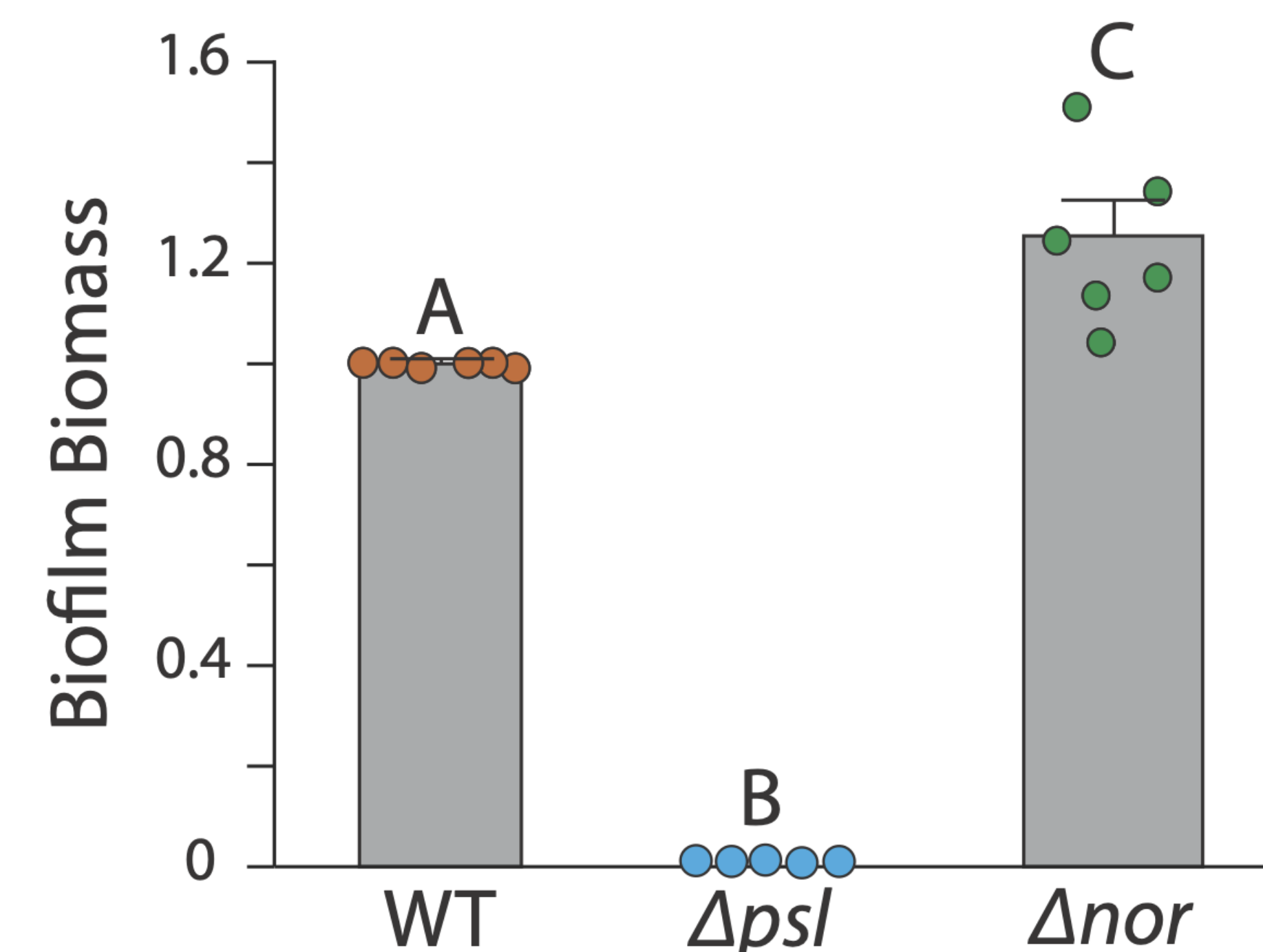
We hypothesize that loss of NOR will induce stress (due to NO accumulation), resulting in increased biofilm formation.

Standard 96-well CV biofilm assay



Does loss of NOR activity increase biofilm formation?

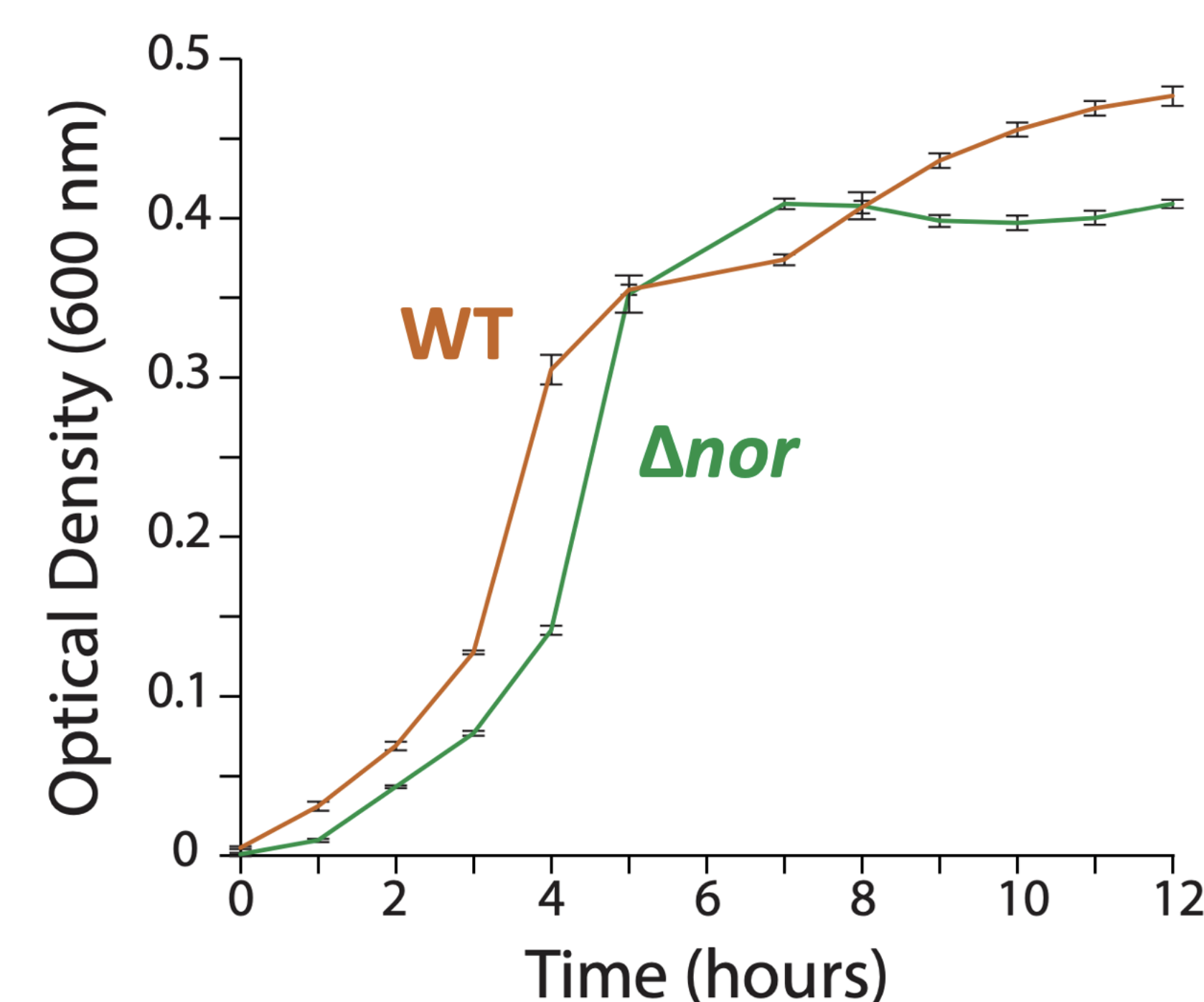
Strains	
WT	Positive control
Δpsl	Negative control
Δnor	Experimental



Biomass normalized to WT. Error bars, SEM (N=6). Letters, statistically different groups ($p < 0.01$; ANOVA with post hoc Tukey)

Δnor produces 1.25x more biofilm biomass

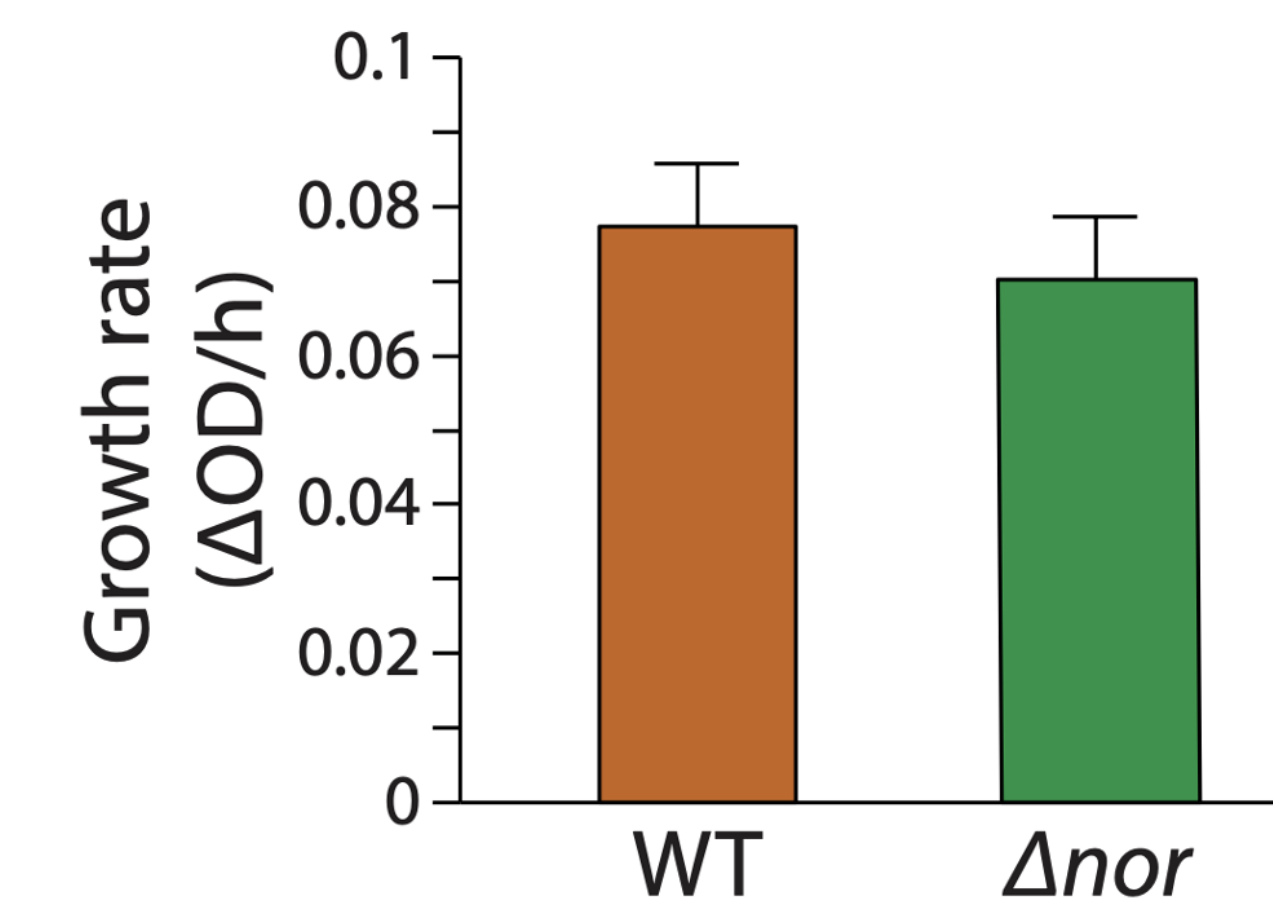
Is the increased biofilm biomass due to increased cell growth?



OD600 was read every 5 minutes during a 12-hour shaking growth curve. Error bars, SEM (N=2, n=24).

Growth of cells themselves are not the cause of increased biofilm biomass

Do Δnor and WT cells have similar doubling times?

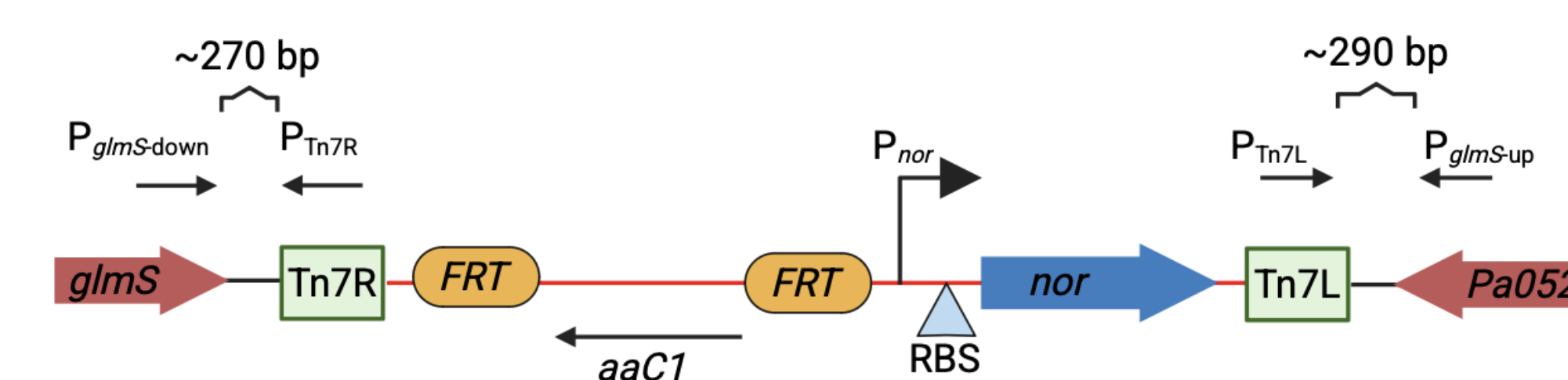


Derived from exponential phase of growth curves. Error bars, SEM (N=2, n=24); No significant difference (unpaired t-test, $p > 0.05$)

Δnor has a similar growth rate to WT

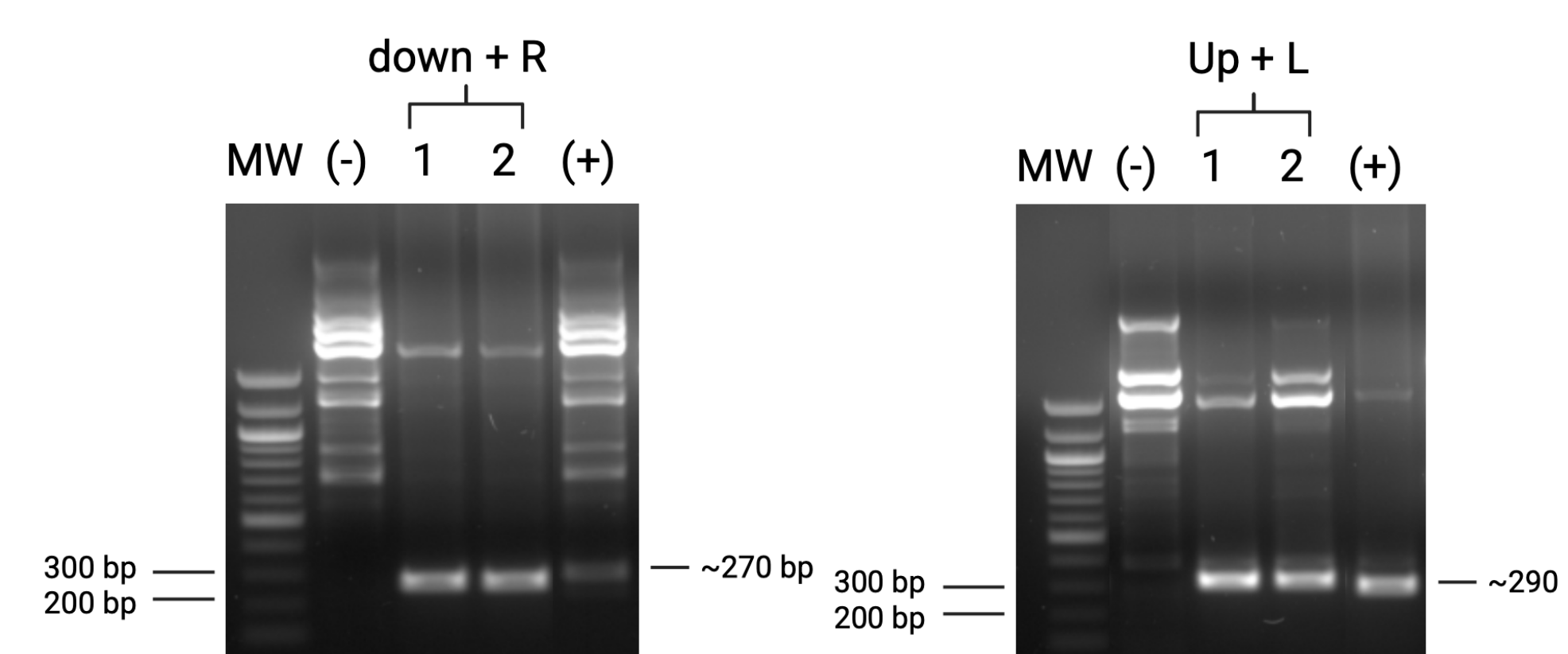
Is deletion of *nor* the cause of increased biofilm biomass?

- Is increased biofilm biomass due to a secondary mutation in the Δnor mutant strain?
- To test, rescue NOR activity by adding a WT copy of NOR back into Δnor mutant strain
- Used miniTn7 system to insert into genome



Schematic showing chromosomal integration of the *nor* rescue cassette at the attTn7 site. Primer pairs $P_{glmS-down} + P_{Tn7R}$ (down+R) and $P_{glmS-up} + P_{Tn7L}$ (up+L) generate ~270–290 bp products to confirm insertion, shown in the PCR verification.

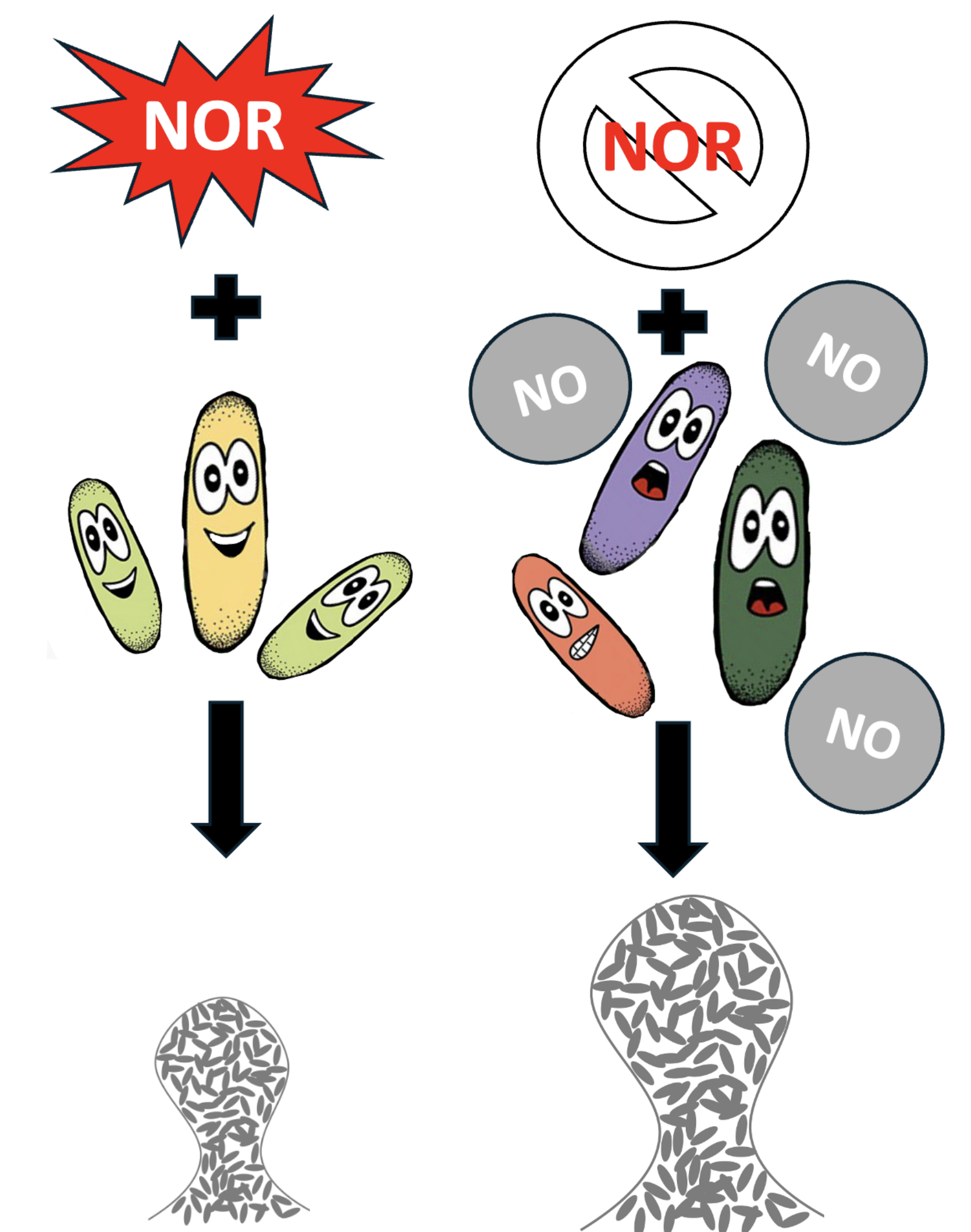
PCR confirmation of *nor* cassette insertion into genome



PCR verification, using $P_{glmS-down} - P_{Tn7R}$ (down+R) or $P_{glmS-up} - P_{Tn7L}$ (up+L), to detect insertions at attTn7. Gel electrophoresis on 1% agarose gel was used to analyze PCR products of 2 clones with the NOR rescue cassette integrated into the chromosome.

Summary

- Loss of NO detoxification significantly increased biofilm formation
- Δnor likely increases residual NO concentrations due to its inability to reduce NO in oxygen-depleted environments
- Rescue strain was successfully created, but more tests need to be done to verify the role of NOR



Future Directions

- Assess intracellular c-di-GMP concentrations of WT and Δnor mutants to understand role of NO on this key biofilm secondary messenger molecule
- Measure number of biofilm cells and quantify individual matrix components to determine which component is increased in Δnor biofilm

Acknowledgements

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