

Effectiveness of *Escherichia coli* Nissle in preventing cariogenic environments caused by *Streptococcus mutans*

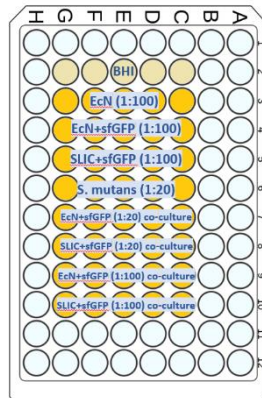
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Introduction: Dental caries are a disease characterized by deteriorated enamel. *Streptococcus mutans* are highly cariogenic oral bacteria that catabolize multiple carbohydrates to secrete acids and polysaccharides that promote bacteria adsorption to the tooth (i.e. biofilm). *Escherichia coli* Nissle (EcN) and its engineered variants are evaluated in their ability to survive against *S. mutans* and inhibit its acid and biofilm production, as eventually they will be delivered to white spot lesions to facilitate remineralization and disinfection.

Methods: Brain Heart

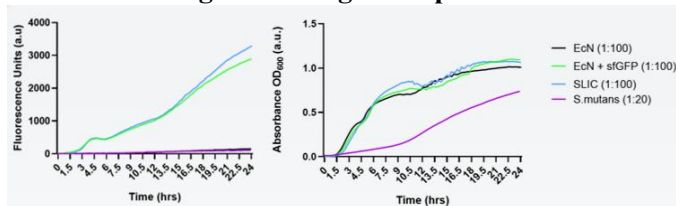
Infusion (BHI) media, EcN, sfGFP-tagged EcN, its self-lysing variant (SLIC), and *S. mutans* were pipetted into single-culture and co-culture wells (n=5) in a 96-well plate. At 37°C, a plate reader (BioTek) records the plate's fluorescence and absorbance values for 24 hr at 15 min increments.



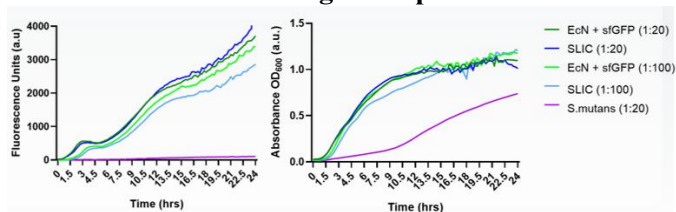
After aspirating the cells and media, the wells are stained with 0.1% crystal violet solution (Sigma). The plate reader reads the absorbance (OD=585 nm). The pH of corresponding Falcon tubes is each measured with a pH probe at 0, 4.5, and 24 hr.

Results:

Single culture growth profiles

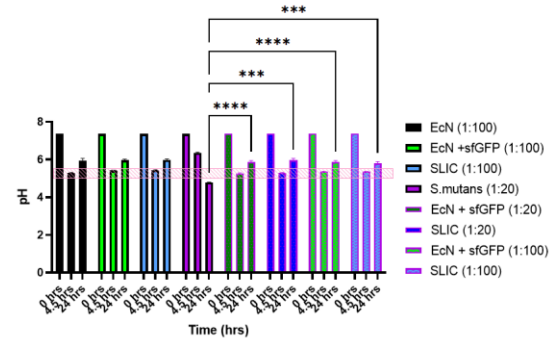


Co-culture growth profiles



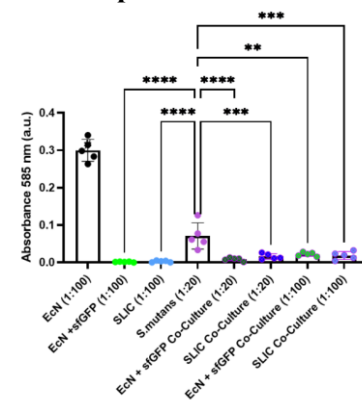
The 1:100 co-cultures' growth profiles resemble those of corresponding single cultures. Oscillations in absorbance occur across all co-cultures after *S. mutans* has attained its exponential growth phase.

pH over time



After 24 hours, *S. mutans* alone drops to a pH of 4.8 while the co-cultures end with a pH above 5.5.

Biofilm deposition after 24 hours



Engineered EcN produced virtually no biofilm. When plated with either engineered EcN, *S. mutans* experiences a significant reduction in biofilm deposition.

Conclusions: SLIC's survival is unimpeded by the presence of *S. mutans*, showing potential as a probiotic capable of releasing assistive proteins through self-lysis. The oscillations in absorbance suggest competition, but this requires further investigation with quantitative measurements of gene expression by qPCR. Engineered EcN shows promise in counteracting *S. mutans*' biofilm deposition and acidity.

References:

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2. Lemos, J. A., Palmer, et al. (2019). The Biology of *Streptococcus mutans*. *Microbiology spectrum*.
3. Lemos, J. A., & Burne, R. A. (2008). A model of efficiency: stress tolerance by *Streptococcus mutans*. *Microbiology (Reading, England)*, 154(Pt 11), 3247–3255.

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