Characterizing Biofilm Dispersion in the Gut Bacterium *Enterococcus faecalis*

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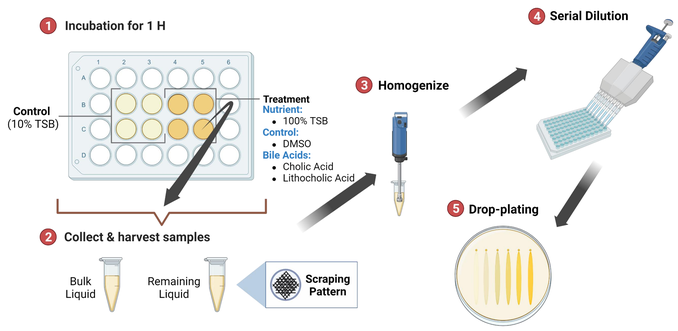
E. faecalis undergoes nutrient-induced biofilm dispersion—alterable by bile acids—and displays antibiotic tolerance.

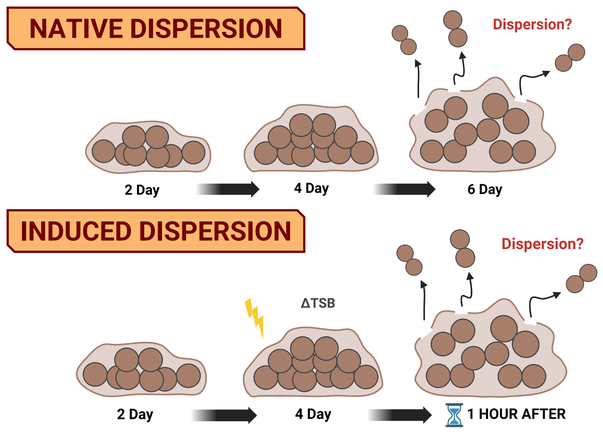
Introduction:

- **Biofilm dispersion** is an important survival mechanism for bacteria where they leave their matrix and return to a planktonic phenotype.
- If dispersion does not occur, bacteria will grow continuously, run out of nutrients, and die, which can be problematic for probiotics in the microbiome.
- **Dispersion is not characterized in *Enterococcus faecalis*** (*E. faecalis*), a gram-positive and nonmotile bacterium commonly found in the human gut. Thus, **our goal was to better characterize biofilm dispersion in this species and investigate the effect of bile acids and exposure to different antibiotics.**

Study Design:

General Biofilm Dispersion Assay:





Long-Term OG1RF Biofilm Formation:

When is steady state reached?

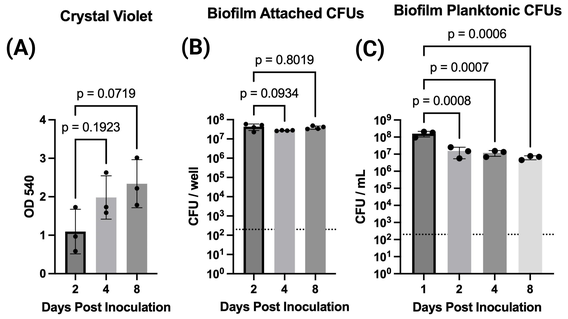


Fig 1. Analysis of native dispersion time course for wild-type OG1RF. Each data point represents the mean of 3-4 individual wells. **(A)** There is a trend towards increased biomass over 8 days as measured by Crystal Violet staining. **(B)** Attached Biofilm CFU levels remain unchanged over the course of 8 days. **(C)** From Day 2 onwards, the level of planktonic OG1RF is unchanged.

OG1RF forms a stable biofilm after **48 hours**, with an overall balanced level of growth and disassembly.

Can confocal microscopy be used to verify steady state biofilm formation?

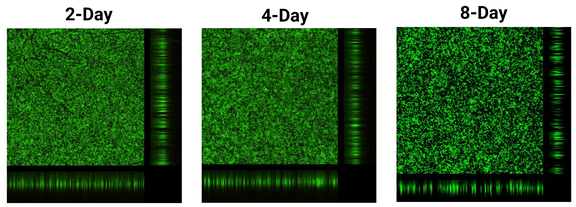
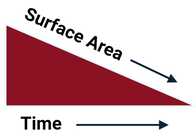


Fig 2. Confocal laser scanning microscopy showing a combined Z-stack of 25 images taken at 0.2-micron intervals at 2, 4, and 8 days of biofilm growth. Cells were stained with SYTO-9.



More exposed substratum (black spaces) on Day 8 than Day 4, which is consistent with fragmentation and disassembly over time despite steady CFU and CV levels.

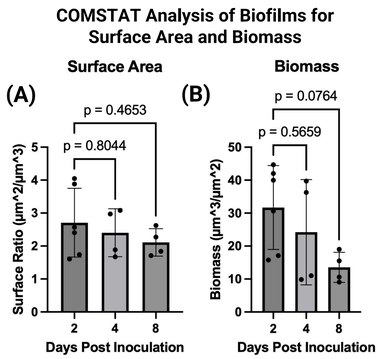


Fig 3. Quantification of biofilm surface area **(A)** and biomass **(B)** over time. Each data point represents the mean of 3-4 individual wells.

Induced Dispersion:

Can dispersion be induced exogenously?

After OG1RF biofilms were grown for 4 days with medium change, the biofilms were treated using a 10-fold increase in nutrients by replacing 10% Tryptic Soy Broth (TSB) with 100% TSB for 1 hour.

Nutrient-Induced Dispersion:

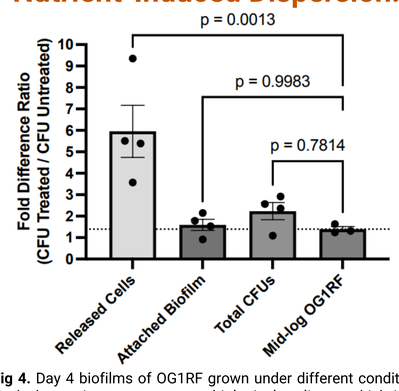


Fig 4. Day 4 biofilms of OG1RF grown under different conditions. Each data point represents one biological replicate, which is the mean of 4 technical replicates.

Dispersion Imaging:

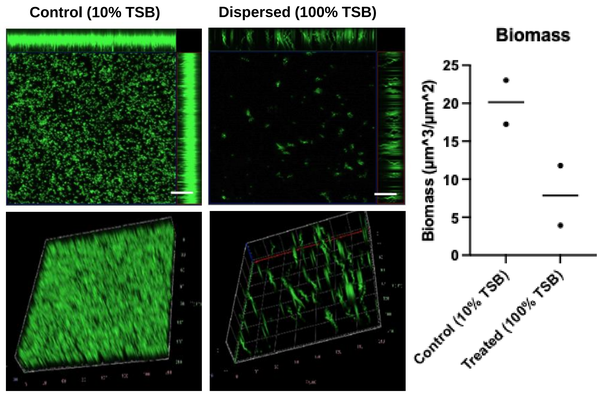


Fig 5. Confocal Laser Scanning Microscopy of Day 4 biofilms uninduced (left) or induced by nutrient step-change (right) for 1 hour followed by fixation with 4% PFA, SYTO-9 staining, and imaging.

Fig 6. Quantification of biomass from 2 independent experiments, each data point represents the mean of 3-4 technical replicate wells.

Cholic Acid and Lithocholic Acid on Nutrient-Induced Dispersion:

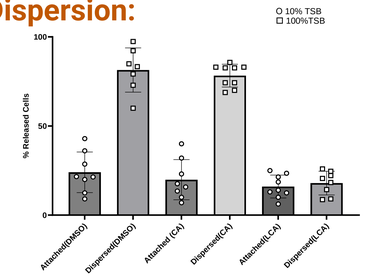


Fig 7. Biofilms grown for 4 days in 10% TSB were either induced (100% TSB) or not (10% TSB). Dispersed cells were separated from attached biofilms, and both were plated after exposure to dimethyl sulfoxide (DMSO) as a control, the primary bile acid cholic acid, or its corresponding secondary bile acid lithocholic acid. Each data point represents the mean of 3-4 wells.

Linezolid and Vancomycin Tolerance:

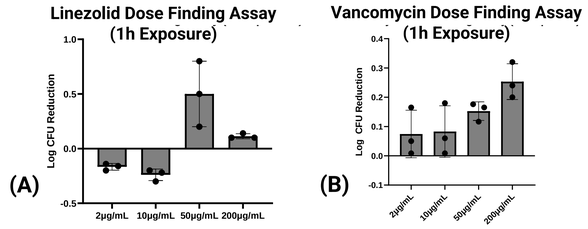


Fig 8. OG1RF mid-log cultures were grown for 18 hours in 100% TSB, shaken at 180 rpm, treated with either the oxazolidinone linezolid **(A)** or the glycopeptide vancomycin **(B)** for 1 hour, washed, and then plated. This experiment was done in triplicate, and each data point represents the mean of 3 technical replicate wells.

Conclusions:

- OG1RF biofilms are capable of dispersion induced by a step-change in nutrients.
- Our data suggest that dispersion may be regulated independently of biofilm formation in response to nutrient step-change.
- Lithocholic acid is able to retain biofilm growth in nutrient-induced dispersion plates while cholic acid maintains dispersion results comparable to DMSO controls.
- Between the linezolid and vancomycin dose finding assays, antibiotic tolerance by OG1RF was displayed only when treated with 50 µg/ml of linezolid, displaying a 0.5 log reduction.

Future Work:

- Investigate dispersion in other transposon mutants suspected to be associated with *E. faecalis* biofilm formation.
- Identify inducers of dispersion and its regulation using multi-omic approaches.
- Investigate how the dispersion response differs in mixed-species biofilms and in the presence of intestinal metabolites.
- Determine tolerance to exposure of different antibiotics.

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References: