

The effect of rugose small colony variants on *Pseudomonas aeruginosa* biofilm dispersion

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Introduction

One of the most common sources of hospital-acquired infections and the leading cause of chronic infections in cystic fibrosis patients is the opportunistic pathogen *Pseudomonas aeruginosa*¹. They exist primarily in biofilms, which are structured, bacterial communities that provide the bacteria with protection and nutrients. Bacteria leave the biofilm during dispersion, the final stage of biofilm development².

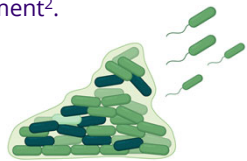


Figure 1. Dispersion³. Bacteria leave the biofilm to colonize other surfaces and/or find better conditions during dispersion. Signals related to external and internal stressors cause subpopulations of the bacteria to leave the biofilm.

Methods



Figure 2. Tube reactor system. The tube reactor system is an apparatus used to study biofilms. A source of nutrients is continually pumped through several lines of tubing where it is emptied into waste carboys. Bacteria are injected into the tubing and allowed to grow over several days until they are ready to be harvested.

Research Question and Results

A unique phenotype called rugose small colony variants, also known as RSCVs, appear in *P. aeruginosa*. They are small, hard colonies that appear wrinkled under a microscope. It is known that they appear in cystic fibrosis patients⁴. My research investigates whether RSCVs disperse from biofilms.

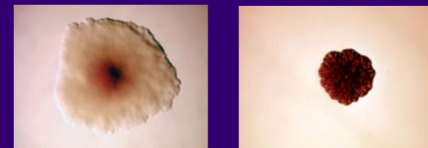


Figure 3. Smooth and RSCV colonies⁴. The image to the left is of a smooth colony and the image to the right is of a RSCV colony. All known information about *P. aeruginosa* biofilms is based on smooth colonies. There is little information known about RSCV colonies. Each panel represents an area that is approximately 5.0 mm × 3.5 mm.

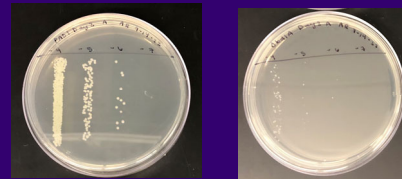


Figure 4. RSCVs do not form in agar and broth solutions. Over the course of five days, bacteria were cultured, serial diluted, and drop plated from agar and broth solutions in an evolution experiment. RSCVs did not form throughout the course of the experiment, indicating that RSCVs may only form in biofilms.

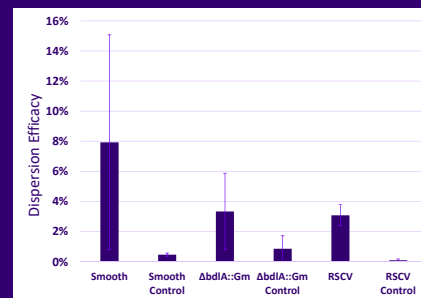


Figure 5. RSCVs release less bacteria. ΔbdIA::Gm forms both smooth and RSCV colonies. RSCV forms only RSCV colonies. ΔbdIA::Gm and RSCV released less bacteria than their parental control, Smooth, when dispersion was induced. This data suggests that RSCVs do not disperse. Error bars represent standard deviation.

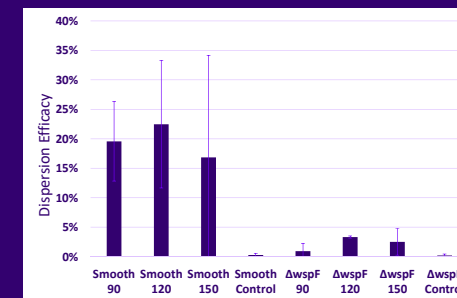


Figure 6. RSCVs release less bacteria in extended dispersion conditions. ΔwspF forms RSCVs. Dispersion was induced with 90-minute, 120-minute, and 150-minute stop flow times. ΔwspF consistently released less bacteria than its parental control, Smooth. This data also suggests that RSCVs do not disperse. Error bars represent standard deviation.

Summary

Dispersion is a growing area of interest as researchers believe it is at this stage of development that we can control biofilms. Biofilms appear everywhere, from the human body to hospital surfaces to the International Space Station. RSCVs are a gain-of-function phenotype that seemingly allow biofilms to attach better to their surfaces and avoid dispersion. It is important to understand how biofilms adapt to their environments as these adaptations may make biofilms even more difficult to control.

Acknowledgements and References

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- ¹ CDC. 2019 Nov 6. *Pseudomonas aeruginosa* Infection.
- ² Rumbaugh K, Sauer K. 2020. Biofilm dispersion. *Nature Reviews Microbiology*.
- ³ Created in Biorender.
- ⁴ Harrison J, et al. 2020. Elevated exopolysaccharide levels in *Pseudomonas aeruginosa* flagellar mutants have implications for biofilm growth and chronic infections. *PLOS Genetics*.