Biofilm formation in a pleiotropic resistant *Pseudomonas fluorescens* strain

Abby Lam, Dr. Jennifer VanderKelen

Center for Applications in Biotechnology

Cal Poly Bailey College of Science & Mathematics

Introduction

Antibiotic resistance is an escalating global health concern, typically associated with the overuse of antibiotics, but other biocides may also contribute to the problem through pleitropic resistance mechanisms.[1] Diacetyl, a fermentation byproduct and food additive, has antimicrobial properties driven by reactive oxygen species that damage proteins, membranes, and DNA.[2]

A *Pseudomonas* strain, 3.2C-A, was previously isolated that can tolerate a high level of diacetyl and also has increased resistance to two antibiotics: chloramphenicol which inhibits protein synthesis and ciprofloxacin acts on DNA gyrase and inhibits DNA synthesis.[3] Thus, this mutant exhibits pleiotropic resistance. For bacteria to tolerate diverse biocides, a generalized resistance mechanism must be involved. Biofilm formation is a common bacterial response that provides protection from environmental stresses. We hypothesize that biofilm formation is part of the adaptive mechanism to diacetyl that also provides resistance to antibiotics.

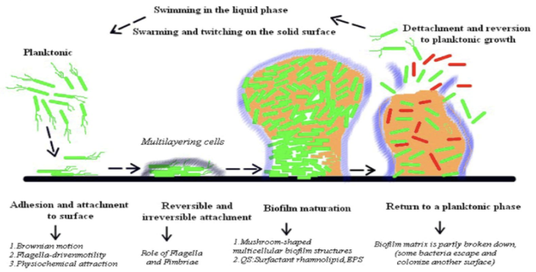

Figure 1. Biofilm lifecycle. Planktonic bacteria attach to a surface, transition from reversible to irreversible adhesion, and form multilayered communities that mature into extracellular polymeric substances-encased biofilms; cells subsequently disperse and return to the planktonic state to colonize new surfaces (Meliani & Bensoltane, 2015).

Methods

To assess biofilm formation:

- Wells of a deep-well block were inoculated with 675 µL of MHB and 75 µL of bacterial culture at a concentration of 1 x 10^7 cfu/mL.
- Wells were covered with a sterile 96-tube semi-skirted PCR plate; the tubes served as peg-like surfaces for biofilm formation (Fig. 2)
- Plates were incubated at 22°C in a humid chamber for 4 days.
- Pegs were rinsed in PBS, heat-fixed at 60°C, stained with 1% crystal violet, and destained in 95% ethanol.
- Concentration of eluted crystal violet in ethanol was determined spectrophotometrically at 570nm.[5]

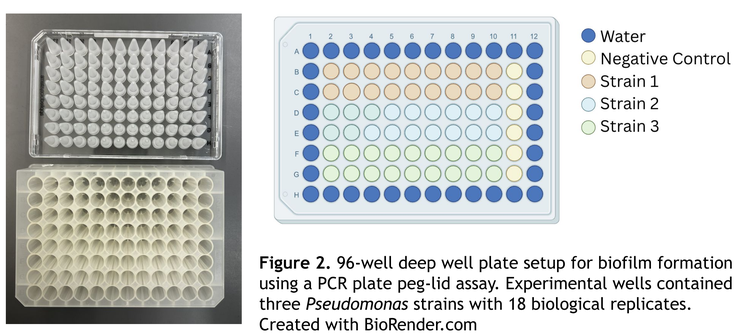

Figure 2. 96-well deep well plate setup for biofilm formation using a PCR plate peg-lid assay. Experimental wells contained three *Pseudomonas* strains with 18 biological replicates. Created with BioRender.com

Results

To assess biofilm formation, wildtype *P. fluorescens* 225, a non-antibiotic resistant but diacetyl adapted strain 3.2C, and the pleiotropic resistant strain 3.2C-A were subjected to a biofilm assay.

Wild type and strain 3.2C exhibited low levels of biofilm formation (mean=0.280 and 0.22 respectively).

Mutant 3.2C-A had higher average biofilm formation (mean= 0.831), but very high variability and extreme values (Fig. 3).

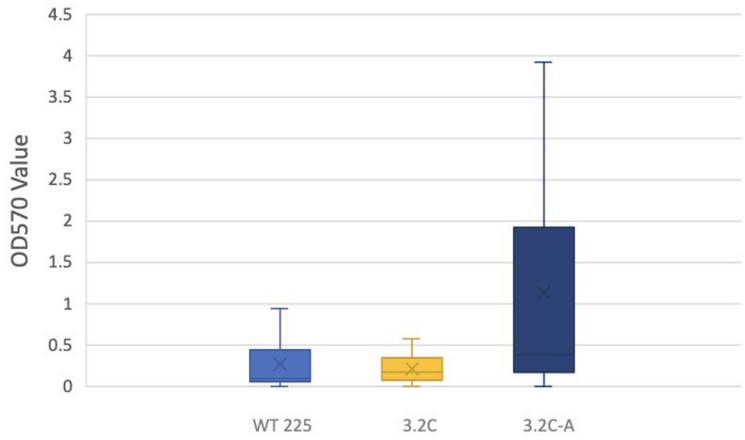

Figure 3. Biofilm formation increased in pleiotropic resistance strain 3.2C-A compared to wild type *Pseudomonas fluorescens* 225, and a non-pleiotropic diacetyl-adapted strain, 3.2C. In each trial, 18 replicates per strain were tested in each of 2 trials.

One caveat to this experiment is that biofilms did not always adhere well to the pegs (Fig. 4). It is possible to see film remaining in the wells after removal of the PCR plate. We are optimizing the protocol to capture all biofilm that is generated.

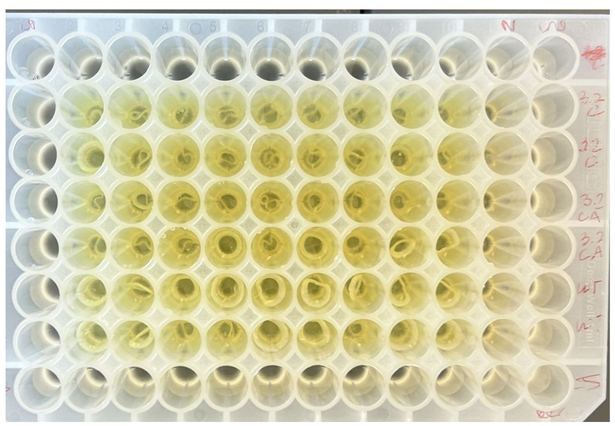

Figure 4. Four days post-inoculation, biofilm growth was observed around the pegs in 96 deep well plate.

Discussion

- The 3.2C-A diacetyl adapted mutant showed a trend toward increased biofilm formation compared to the wild type and non-antibiotic resistant diacetyl adapted strain (3.2C).
- Variability in biofilm formation and adherence to the pegs both within and across trials was substantial. However, the 3.2C-A mutant was the only strain with very high levels of adhered biofilm in some replicates suggesting the 3.2C-A biofilm might be stickier.
- 3.2C-A contains 8 mutations which may be contributing to the biofilm formation and the resulting biocide and antibiotic tolerance.
- Diacetyl exposure can select for genetic adaptations that contribute to pleiotropic resistance. Biofilm formation, and/or differences in adherence or composition could be mediating the pleiotropic resistance.

Future Directions

- Increase the number of trials using the PCR-plate deep well system.
- Test additional pleitropic mutants.
- Optimize the time of biofilm formation.
- Research relationship between mutated genes and biofilm formation.

References

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