Under Selection: Biofilm Adaptation Places *yciR* at the Intersection of Quorum Sensing and Cyclic-di-GMP Metabolism

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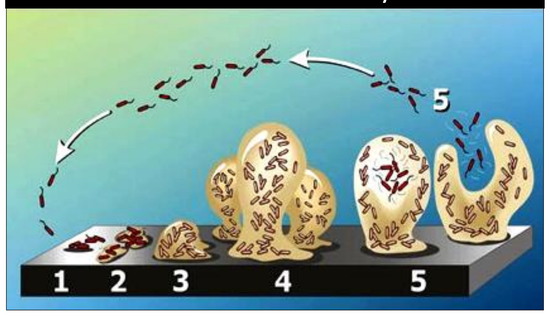
Introduction

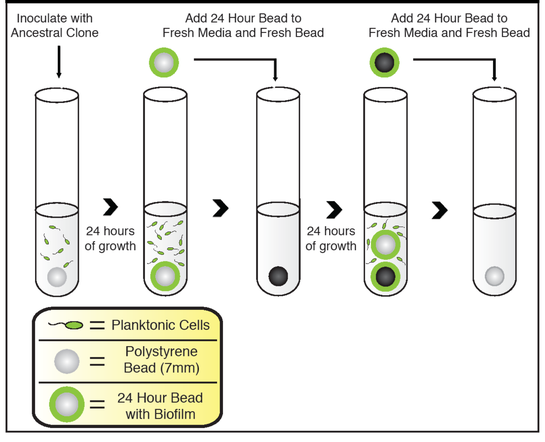
During adaptation in the close quarters of biofilms, different selective pressures for stress tolerance or polysaccharide production can favor mutants inhabiting different ecological niches. Similarly, adaptation during chronic infections by pathogenic bacteria often produces heterogeneous populations associated with worse patient outcomes. One such pathogen, *Burkholderia cenocepacia*, appears to rely upon environmental cues to transition between planktonic and biofilm lifestyles during colonization and persistence within the lungs of individuals with cystic fibrosis. At the forefront of this transition are the quorum sensing systems that promote bacterial communication through perception of secreted factors.

We conducted a long-term experimental evolution with *B. cenocepacia* that selects for daily cycles of adherence and dispersal. Sequencing of replicate populations revealed the persistence of lineages with independent mutations in the *yciR* gene, which physically and enzymatically links the perception of the BDSF quorum sensing molecule with synthesis of c-di-GMP, a secondary messenger that induces biofilm formation.

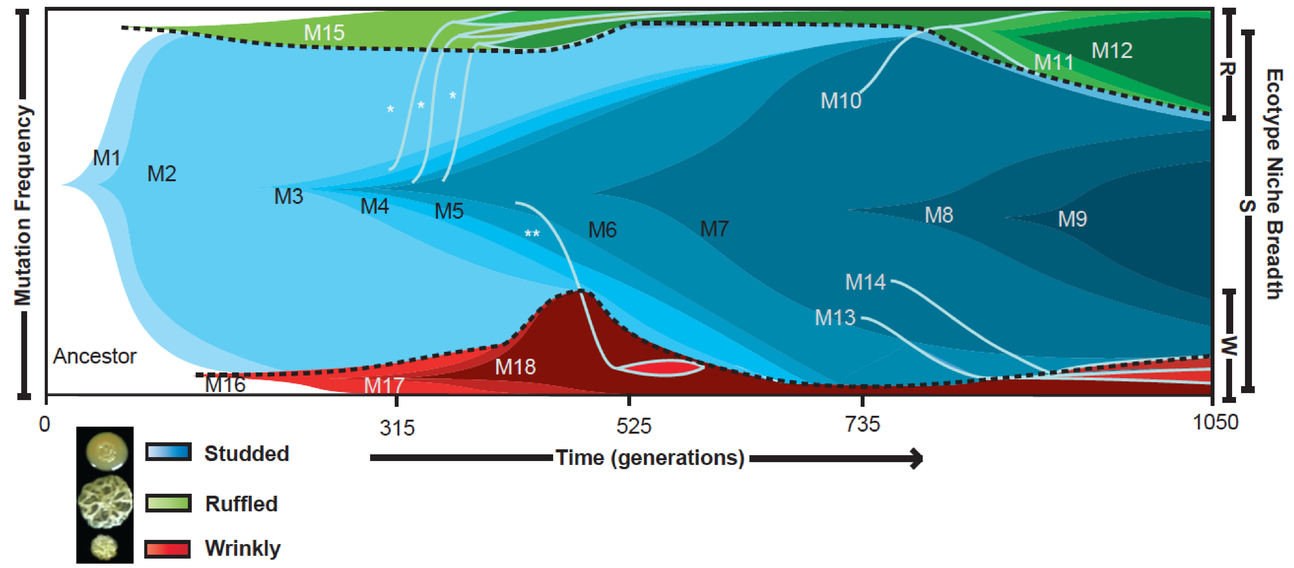
The Natural Biofilm Cycle



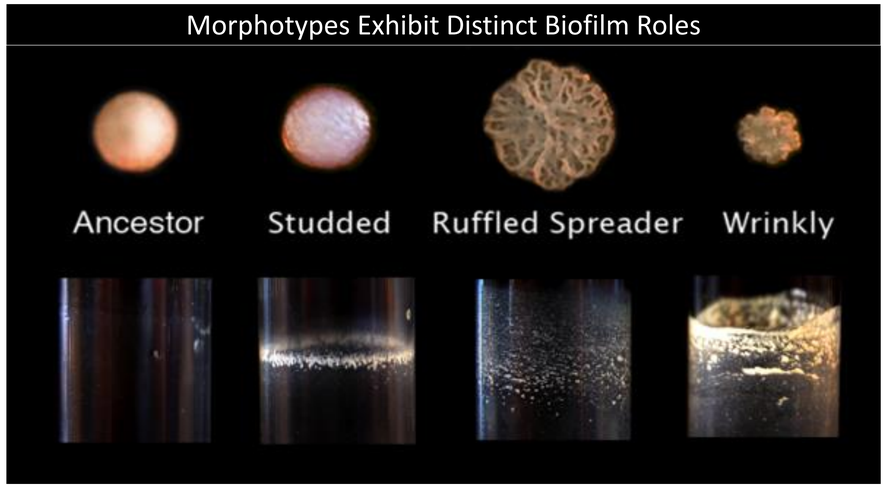
Experimental Evolution in the Bead Model



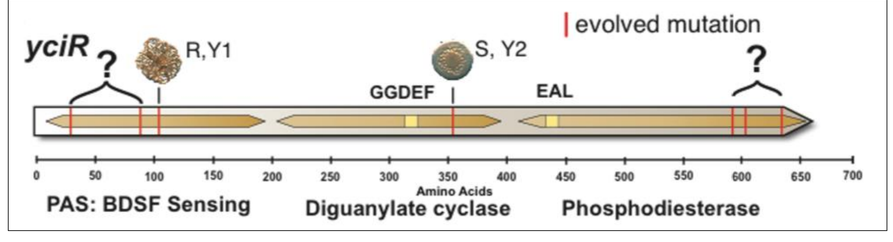
Analysis of Population Genomics Reveals Ecological Diversification and Evolutionary Succession



Morphotypes Exhibit Distinct Biofilm Roles



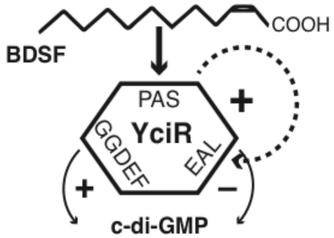
Mutations are Detected throughout *yciR* Domains



c-di-GMP Levels Vary in Mutants

Genotype	c-di-GMP level 10 hours	18 hours
yciR A106P	++	+
yciR Y355D	+	++
Δ*yciR* Δ*rpfF*	+++	++

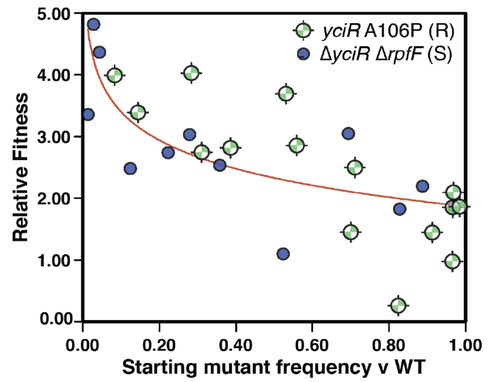
Proposed Model



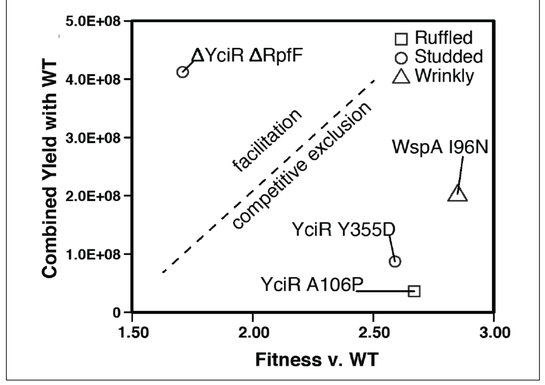
Hypothesis

BDSF is a dispersal signal that can be cheated by mutations in *yciR*

yciR Mutants Display Negative Frequency Dependent Fitness



Ecological Mechanism of Fitness Advantages Varies in *yciR* Mutants



Current Research

We have generated isogenic mutant strains to study the individual *yciR* mutant alleles in isolation.

To examine how each allele impacts the timing of attachment and dispersal, we will assay the isogenic mutants for:

- Intracellular levels of c-di-GMP
- Biofilm production
- Fitness in planktonic and biofilm environments

Using RNAseq we will determine the *yciR* regulon in the presence and absence of BDSF.

Our collaborators are currently performing:

- Functional studies to examine how the *yciR* alleles impact DGC and PDE enzymatic activities
- Structural studies to examine how the *yciR* alleles impact BDSF binding and conformation changes