Quantifying Biofilm Formation in Evolved Planktonic and Biofilm-Forming Populations of *Burkholderia cenocepacia* in Carbon-Limited Media

Katherine Jemian, Heather Blasius, and Dr. Caroline Turner
Department of Biology, Loyola University Chicago, Chicago, Illinois

LOYOLA UNIVERSITY CHICAGO
Preparing people to lead extraordinary lives

Abstract

Burkholderia cenocepacia, a pathogenic bacterium to patients with cystic fibrosis, forms a thick biofilm which aids adherence to the lungs' thickened mucosa. This debilitates patients' ability to breathe effectively, enhancing the risk of chronic illness and mortality. Bacterial populations were established from a single population isolate and experienced approximately 500 generations of evolutionary selection in carbon-limited media for biofilm and planktonic-selected biofilm growth. In the lab, we quantified the amount of biofilm produced by evolved populations of *Burkholderia cenocepacia* using spectroscopy and calculated the analysis of variance. We found a significant biofilm growth difference between planktonic and biofilm-forming populations.

Questions

- Did environmental selection occur when a single population isolate was exposed to different conditions that would promote biofilm production?
- To what degree of biofilm growth did these evolved populations achieve when compared with planktonic growth over the same period?

Introduction

- Cystic Fibrosis: selective advantage for bacterial colonization and mucus accumulation on lung epithelium.[1,2]
- *Burkholderia cenocepacia*: opportunistic and most abundant pathogenic species of the *B. cepacia* complex in humans with Cystic Fibrosis.[3,4]
  - Biofilm helps mucosal barrier adherence, which is achieved through quorum sensing.[5]
  - Selective pressures in the lung contribute to chronic disease, worsen prognoses, increases risk of death.[1,2,4]
    - Oxygen limitation, host defenses, nutrient availability, antimicrobial therapies.[2]
    - Bacterial transcriptional reprogramming drives resistance.[2]
  - Increased intrinsic and acquired high antibiotic resistance, cytotoxins diminishes treatment options, increasing morbidity.[1,6,7]

Results

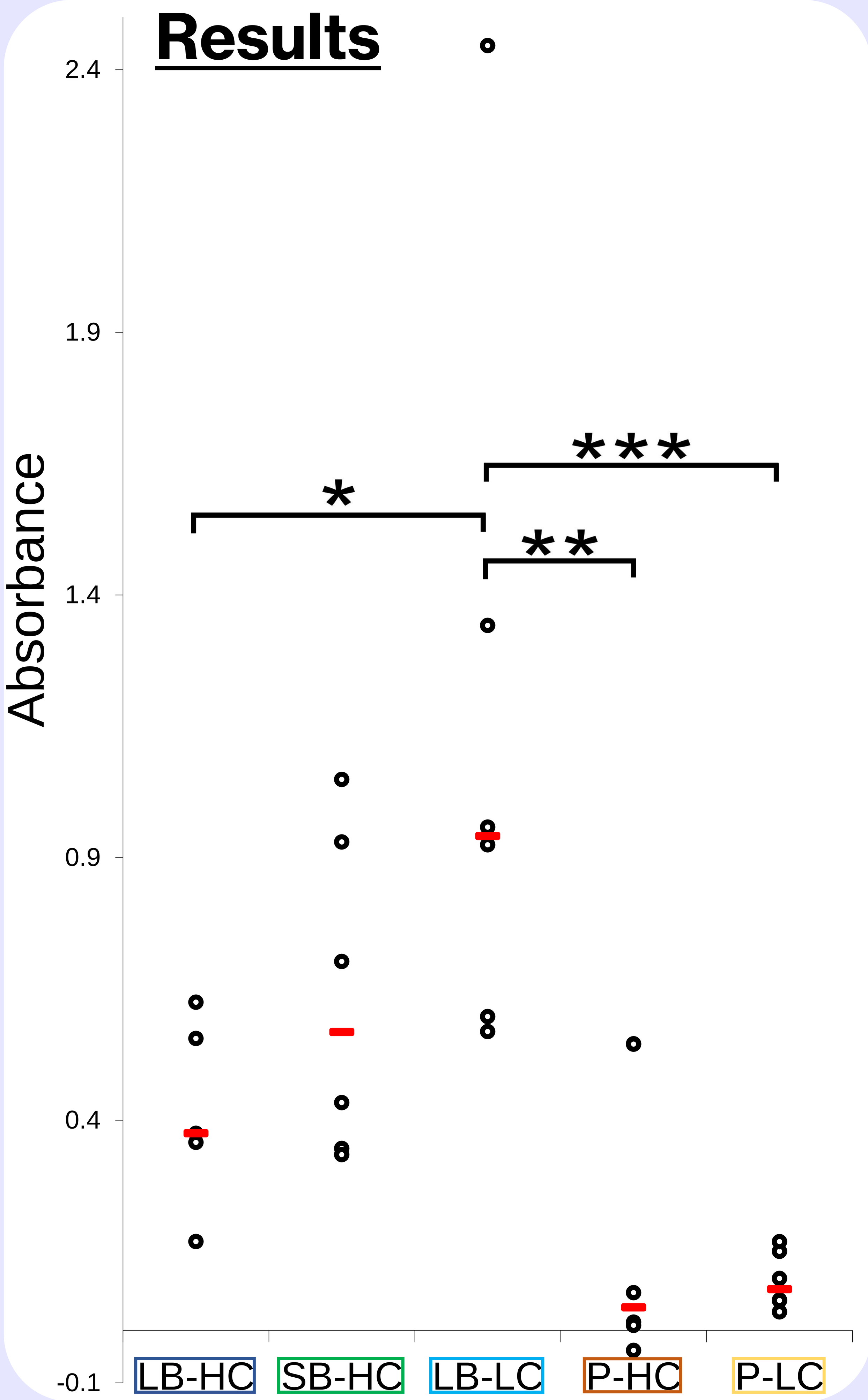

Figure 1. Subtracted Absorbance Values of Evolved Populations. Absorbance values are the average of four replicate populations recorded at 590 nm. Significantly different populations include (Tukey's (p<0.05) value):

- LB-HC-LB-LC (0.0320265)
- P-HC-LB-LC (0.0019365)
- P-LC-LB-LC (0.0006462)

- **LB**: Large Bead
- **SB**: Small Bead
- **P**: Planktonic
- **HC**: High Carbon
- **LC**: Low Carbon

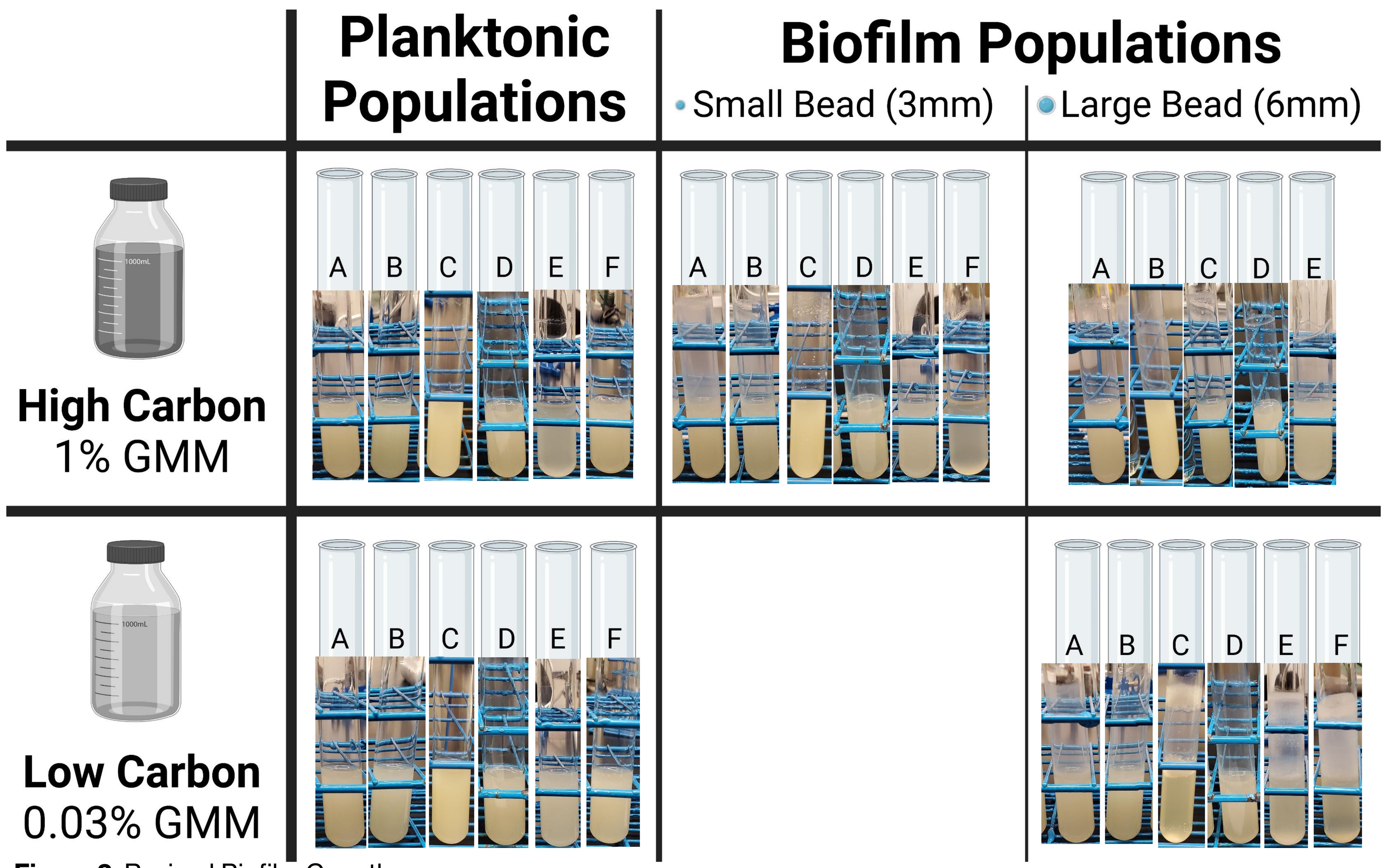

Figure 2. Revived Biofilm Growth.
- Planktonic populations had no distinguishable biofilm on the walls of the test tube (P-HC; P-LC). Some cultures had transparent biofilm with a light film (LB-HC; SB-HC: C, E, F; LB-LC: B, D), where others had more translucent, milky film (SB-HC: A, B, D; LB-LC: A, C), or opaque growth (LB-LC: E, F).
- Superior ring biofilm growth varied between populations. Some had a very faint line (LB-HC: A, C, D; SB-HC: C, E, F; LB-LC: A, D), and others had thick cloud biofilm (LB-LC: C, E, F).[9]

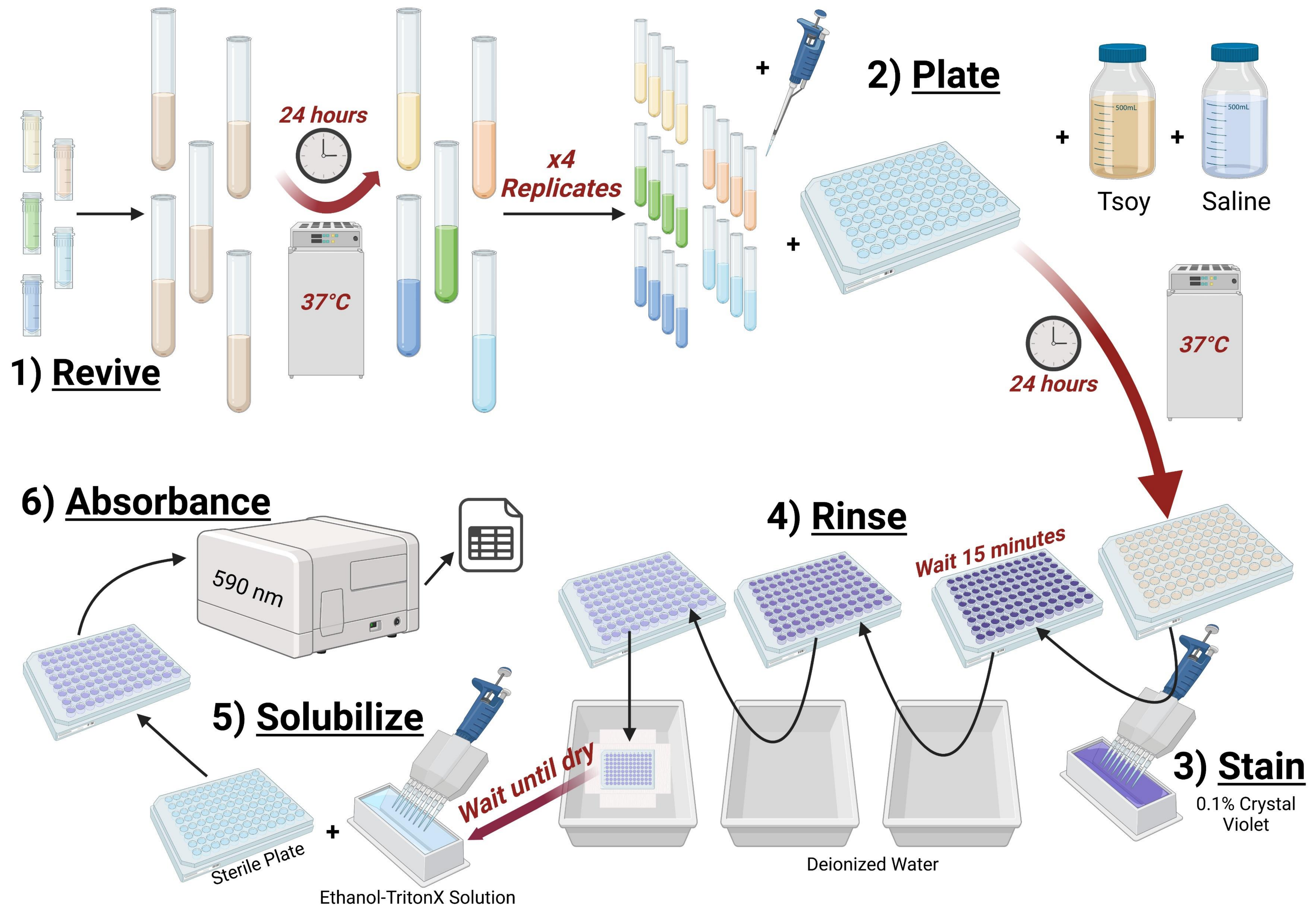

Figure 4. Staining Biofilm Evolved Population. We revived frozen *B. cenocepacia* and inoculated Trypticase Soy nutrient-rich broth media. We analyzed and collected absorbance data on Excel and RStudio using the Analysis of Variance (ANOVA) multifactorial t-test with a post-hoc Tukey's test to analyze significant differences in biofilm growth.[9]

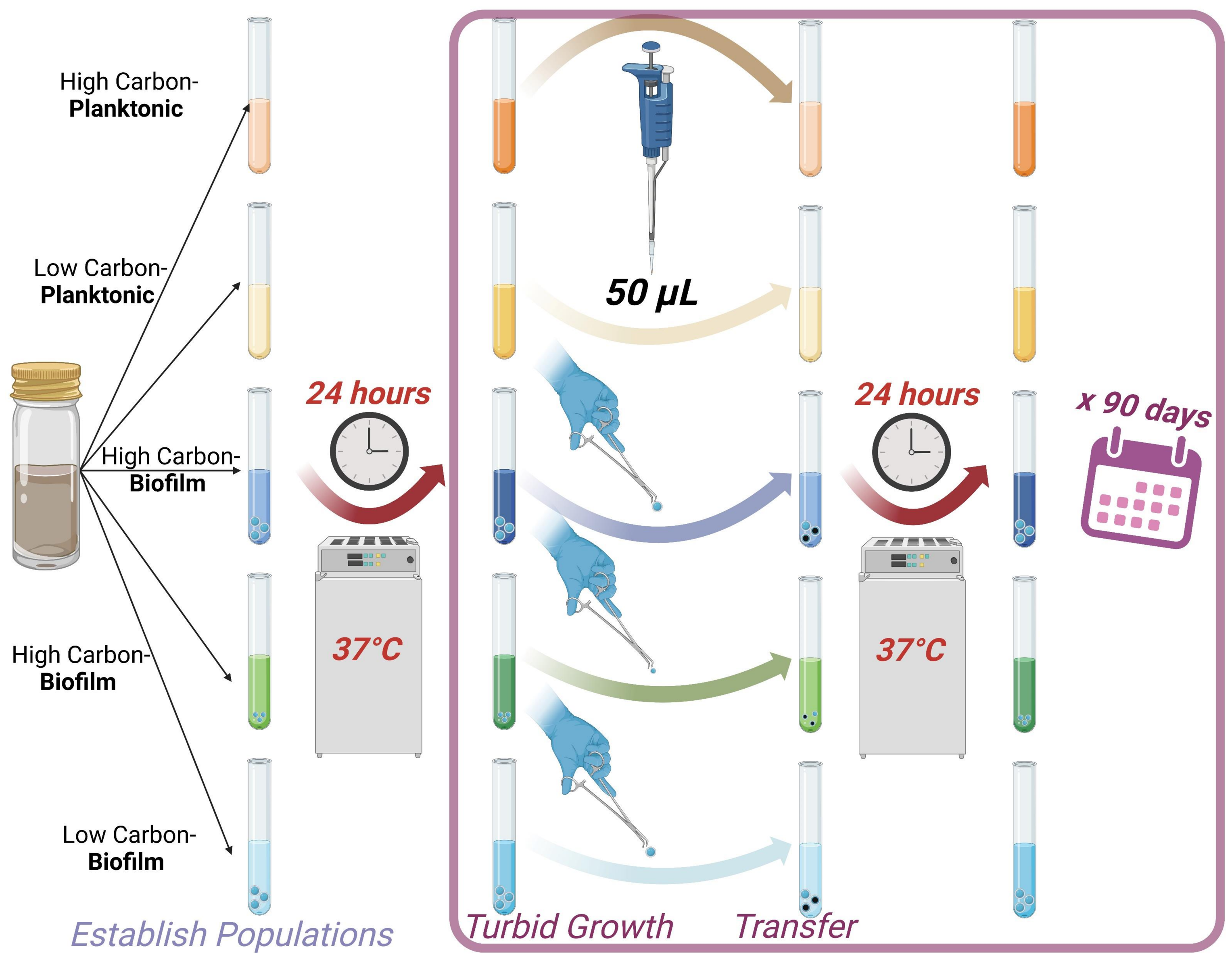

Figure 3. Biofilm and Planktonic Selection. Populations were raised in Galactose Minimal Media. After 90 days, 1mL of each population was preserved and frozen in an -80°C freezer in a solution of 8% DMSO as a cryoprotectant.[8,9]

Conclusion

- Planktonic selected populations formed least amount of biofilm.
  - Correlates to loss of function mutations in critical biofilm formation genes, indicating tradeoffs in energy consumption and proliferation.
- Similar biofilm growth between populations evolved in parallel, some with variable growth.
  - Common environmental pressure indicates parallel evolution.
  - Radiative adaptive diversification: independent generalist strain specialization to adapted biofilm-forming strains.
- Large Bead-Low Carbon biofilm growth significantly different than:
  - Planktonic-High Carbon: bead selection promotes biofilm growth.
  - Large Bead-High Carbon: carbon limitation selects for biofilm growth more strongly than biofilm selection.
  - Planktonic-Low Carbon: biofilm selection combined with carbon limitation most strongly drives biofilm growth.

References:

1) Scoffone VC, et al. "*Burkholderia cenocepacia* Infections in Cystic Fibrosis Patients: Drug Resistance and Therapeutic Approaches." *Frontiers in Microbiology*, vol. 8, p. 1592, 22 August 2017.
2) Mira NP, et al. "Genomic Expression Analysis Reveals Strategies of *Burkholderia Cenocepacia* to Adapt to Cystic Fibrosis Patients' Airways and Antimicrobial Therapy." *PLoS ONE*, vol. 6(12), e28831, 2011.
3) Coenye T, et al. "Recovery of *Burkholderia cenocepacia* strain PHDC from cystic fibrosis patients in Europe." *Thorax*, vol. 59, p. 952-954, 2004.
4) Traverse CC, et al. "Tangled bank of experimentally evolved *Burkholderia* biofilms reflects selection during chronic infections." *PNAS*, vol. 110(3), p. E250-E259, 27 December 2012.
5) Suppiger A, et al. "Two quorum sensing systems control biofilm formation and virulence in members of the *Burkholderia cepacia* complex." *Virulence*, vol. 4(5), p. 400-409, 1 July 2013.
6) Schoch CL, et al. *NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford). 2020: baaa062.* PubMed: 32761142 PMC: PMC7408187.
7) Burkholderia cepacia: Organism Overview, *NIH, Genome*: ID: 10703.
8) Turner CB, et al. "Parallel genetic adaptation across environments differing in mode of growth or resource availability." *Evolution Letters*, vol. 2(4), p. 355-367, 4 August 2018.
9) Created with biorender.com.