



Research article

Uncovering the phenotypic and transcriptional characteristics of biofilm-released cells in *Pseudomonas aeruginosa*

Jason Li^{1,†}, Larry Ayiku^{1,2,†}, Benjamin Larson^{1,2,†}, Agam Patel^{1,2}, Dylan Adams^{1,2}, Patrick Moran¹, Samantha Burgan^{1,2}, Esha Shah¹, Anthony Bruno^{1,2}, Jaclyn Printz^{1,2}, Tevin Flom^{1,2} and Whitney K. Redman^{2,*}

¹ Department of Biological Sciences, Binghamton University, Binghamton, NY, United States

² First-year Research Immersion Program, Binghamton University, Binghamton, NY, United States

* **Correspondence:** Email: wredman@binghamton.edu.

† These three authors contributed equally.

Supplementary

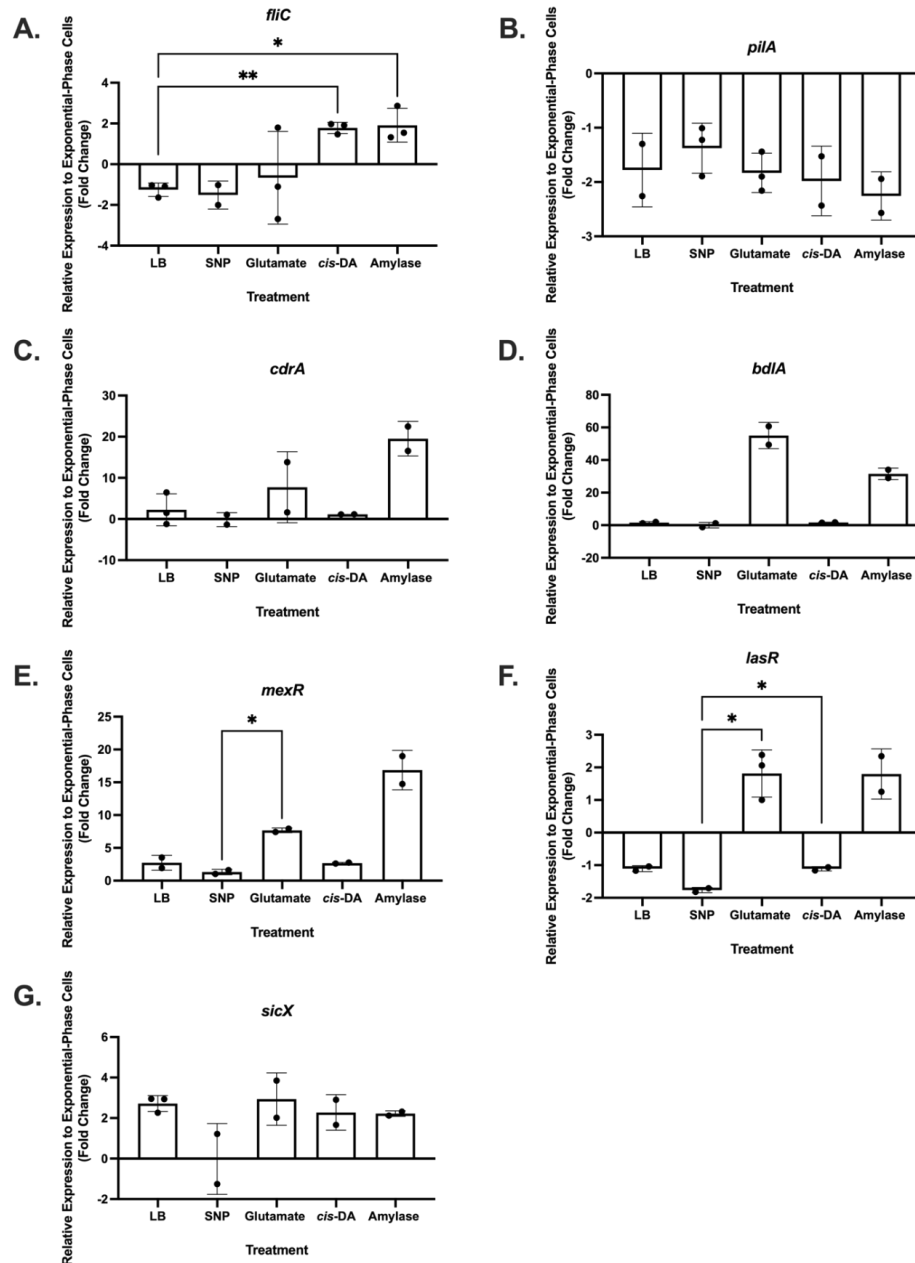


Figure S1. Gene expression of biofilm-released *Pseudomonas aeruginosa* cells normalized to exponential-phase controls. RT-qPCR analysis of gene expression in PAO1 cells released from 2-day biofilms following treatment with LB (control), SNP, glutamate, cis-DA, or amylase. Released cells were collected, RNA was extracted, and relative gene expression was calculated using the $\Delta\Delta C_t$ method normalized to untreated exponential-phase cells. Panels represent relative gene expression for (A) *fliC*, (B) *pilA*, (C) *cdrA*, (D) *bdIA*, (E) *mexR*, (F) *lasR*, and (G) *sicX*. Statistical significance was determined using Brown-Forsythe and Welch ANOVA followed by Games-Howell multiple-comparisons testing with multiplicity-adjusted P values. Mean $\pm$ standard deviation is shown (* $p < 0.05$; ** $p < 0.01$). $n = 2-3$ independent biological replicates/group, with each biological replicate analyzed in technical duplicate and technical duplicate values averaged prior to statistical analysis.

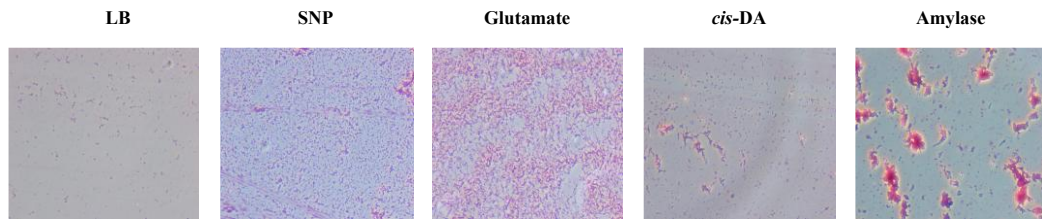


Figure S2. Microscopy of released-cell populations following treatment with various releasing agents shows distinct morphological differences. The micrographs above show the resulting cell populations in the bulk supernatant after treatment with 1 mL of full-strength LB, 500 μ M SNP, 18 mM glutamate, 310 nM *cis*-DA, or 500 U/mL amylase. The LB-treated sample shows a small abundance of free cells in the liquid. Conversely, SNP and glutamate treated samples show a high abundance of individual free cells with minimal matrix-bound aggregates of biofilm cells. *cis*-DA treatment overwhelmingly results in free cells with a few aggregates and visible lysed cells. Amylase shows the lowest abundance of free cells with cells being arranged in matrix-bound aggregates of cells.

Table S1. RT-PCR primers.

Gene	Function	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>rho</i>	Transcription termination factor	ATGAAGATGGAAGCCGGCAA	TGCCGGTACCCCTGAATTCC
<i>fliC</i>	Motility and attachment	CGAACTGACCCGTATCTCCG	TCGTTGATGCTGGCAGTGAT
<i>pilA</i>	Motility and attachment	ACTGATGATCGTGGTTGCGAT	TCGGAGTGAACATCGGATCC
<i>cdrA</i>	Biofilm formation	GCCCAGGTGGAGATGTTCAA	AGCTGAGGGTGTAATTGGCC
<i>sicX</i>	Acute to chronic infections	GAGTGGAAACGTGCGGAGAG	GTTGCTCGTCGTTTTCCACC
<i>bdlA</i>	c-di-GMP levels and dispersion	ACCAGGAACACGAGATGCAG	TGACAGTTCGCCGATGTTCA
<i>lasR</i>	c-di-GMP levels and dispersion	GGCCTTGTTGACGGTTTTTC	TTGCTGACCGGATGTTTCGAA
<i>mexR</i>	Drug resistance	GAAC TACCCCGTGAATCCCG	TTGCTGACCGGATGTTTCGAA



AIMS Press

© 2026 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0>)