

Putrescine and its metabolic precursor arginine promote biofilm and c-di-GMP synthesis in
Pseudomonas aeruginosa

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Supplemental Figures and Tables

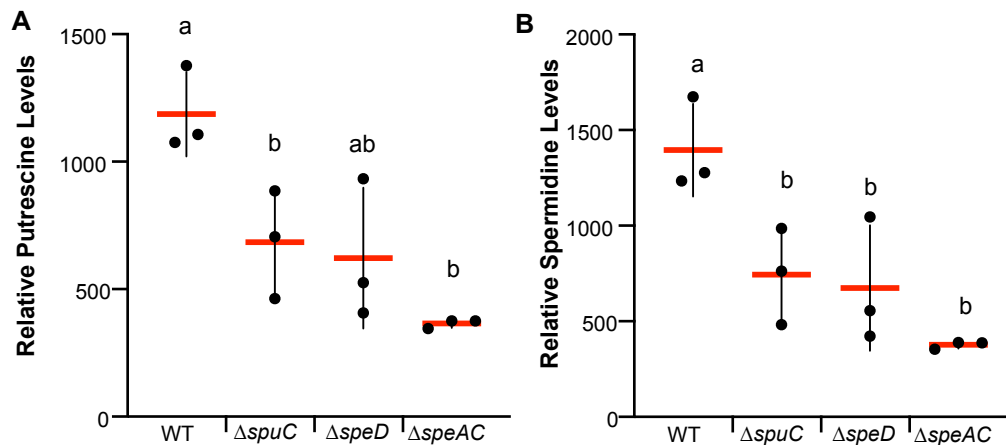


Figure S1. Mutants in predicted polyamine catabolism and biosynthesis genes have reduced levels of putrescine and spermidine. *P. aeruginosa* PAO1 $\Delta spuC$ (putrescine catabolism), $\Delta speD$ (spermidine biosynthesis), and $\Delta speAC$ (putrescine biosynthesis) mutants were grown in M63 media without exogenous arginine or putrescine. Targeted metabolomics was used to quantify putrescine or spermidine levels. All 3 mutants had significant reductions in both spermidine and putrescine compared to wildtype cells. Mean \pm sd is shown; letters indicate genotypes with significantly different levels of polyamines by ANOVA and Tukey's HSD.

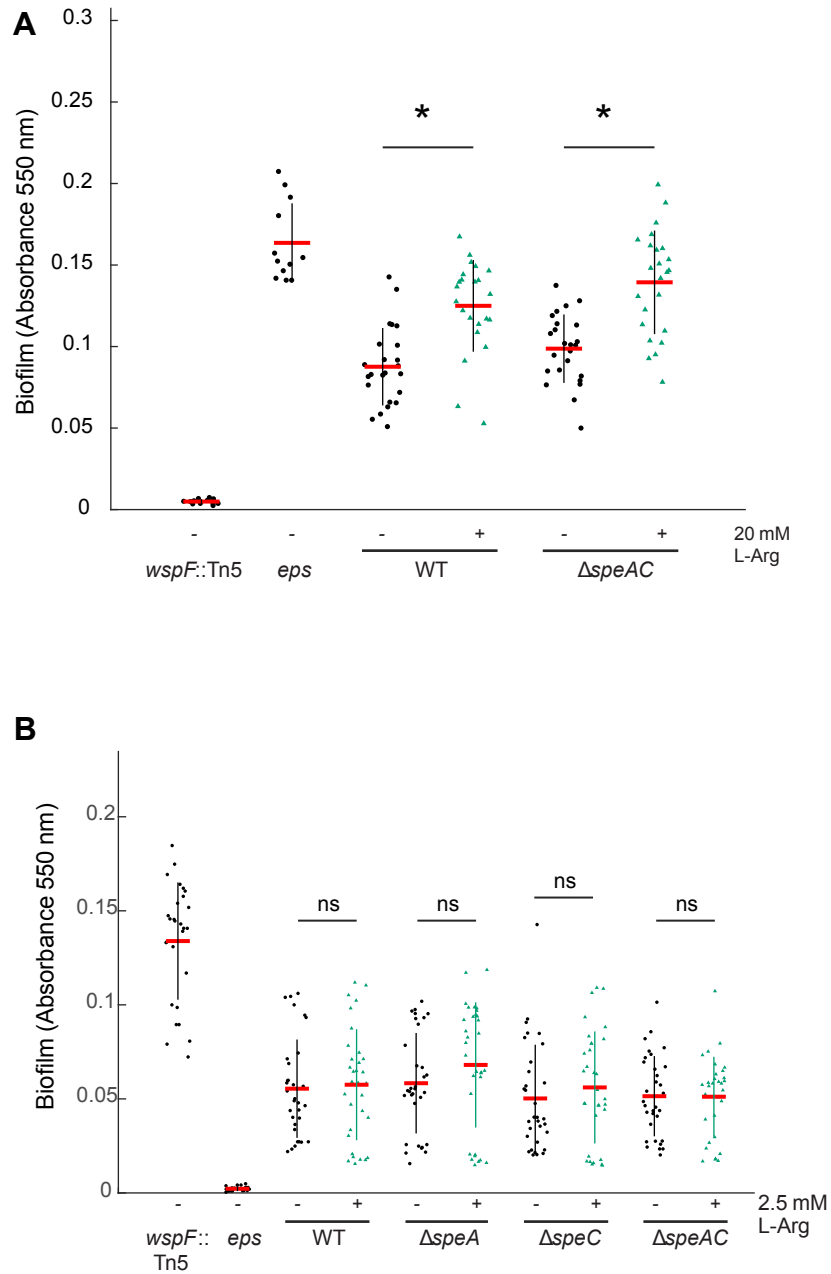


Figure S2. L-arginine robustly promotes biofilm formation independent of SpeA and SpeC. **A)** PAO1 or the *speAC* mutant were treated with 20 mM L-arginine or 20 mM of KCl as a control for the chloride ions in L-arginine HCl salt. * indicates $p < 0.0001$ by student's t-test. Error bars represent standard deviation. Data points show all technical replicates from 3 biological replicates. **B)** Wild-type *P. aeruginosa* PAO1 and Δ *speA*, Δ *speC*, and Δ *speAC* mutants were treated with 2.5 mM L-arginine hydrochloride. At this concentration, L-arginine does not induce biofilm formation in any of the genetic backgrounds, including the L-arginine-accumulating Δ *speA* and Δ *speAC* strains. Error bars represent standard deviation. Data points show all technical replicates from 4 biological replicates.

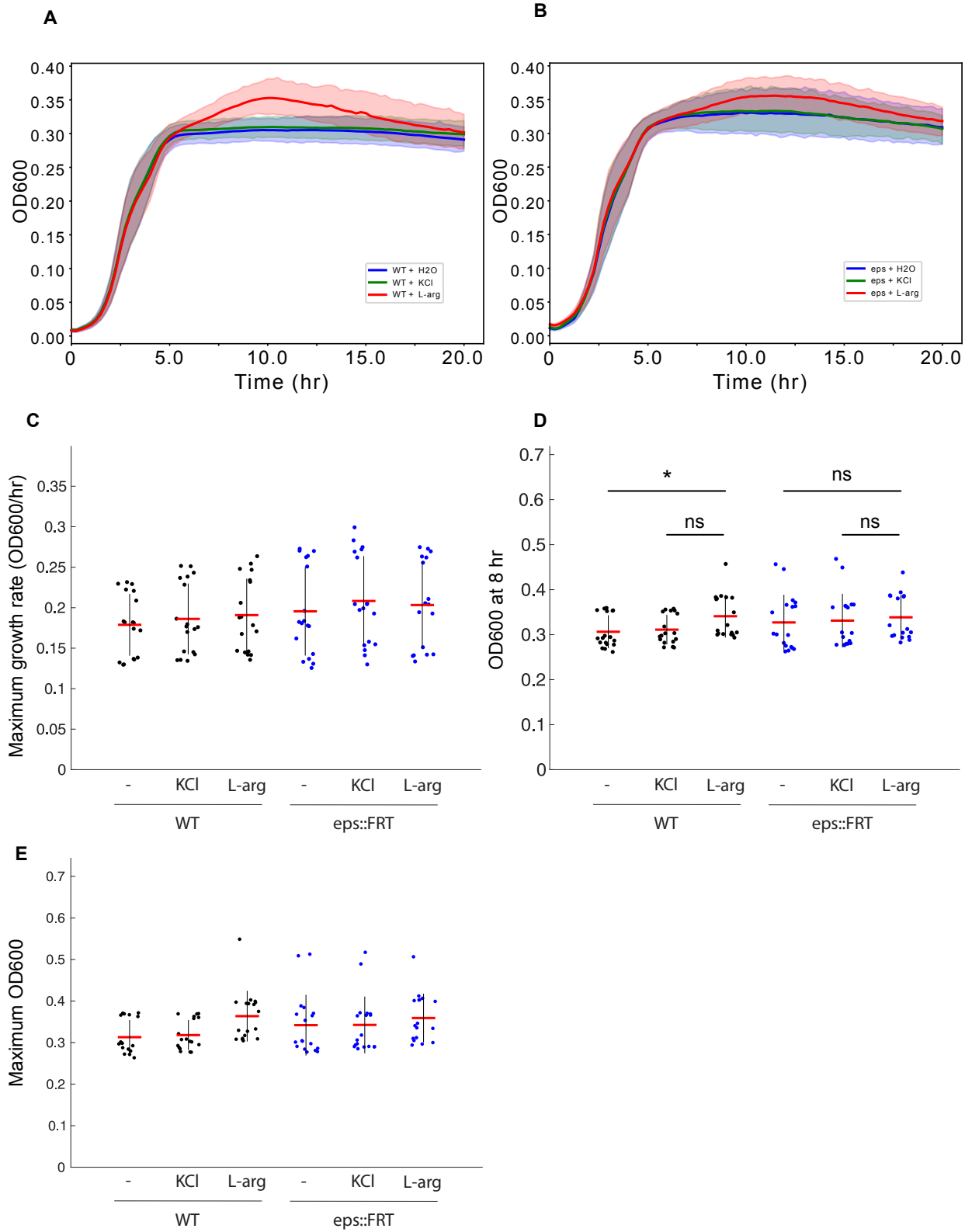


Figure S3. Addition of exogenous L-arginine leads to a slight increase in bacterial density in wild-type but not in the $\Delta eps::FRT$ strain. A-B) Growth curves of *P. aeruginosa* PAO1 WT and $\Delta eps::FRT$ strain in M63 supplemented with 20 mM L-arginine HCl, 20 mM KCl, or equal volume H₂O (error bands represent 95% confidence intervals, $n \geq 17$ from 3 biological replicates). **C)** L-arginine HCl supplementation does not affect the maximum growth rate of either wild-type or the $\Delta eps::FRT$ strain. **D)** While the L-arginine HCl supplementation increases the maximum of OD₆₀₀ at 8 hr (D), this increase is dependent on exopolysaccharide biosynthesis (* $p < 0.05$ by student's t-test, $n \geq 17$ from 3 biological replicates). **E)** The addition of L-arginine HCl or KCl does not significantly alter the maximum growth rate of either the WT or $\Delta eps::FRT$ strain ($p = 0.2477$ by one-way ANOVA, $n \geq 17$ from 3 biological replicates).

Table S1 Primers used in this study

Names	Restriction sites	Sequences (5' → 3')
speA-UpF	HindIII	TTTAAAAGCTTCGCCTGTCCGGCGACG
speA-UpRc		GCTAGCCAGGCGCGGTGATCTC
speA-DnF		GCGCCTGGCTAGCCCGTCG
speA-DnRc	XbaI	CAATTTCTAGAGGCCCTGGTGGCGTTC
speC-UpF	HindIII	TTTAAAAGCTTCGCCCAGGTGACCCAG
speC-UpRc		CGACTGCGGGTTGGGACTCCCAATG
speC-DnF		CAACCCGCAGTCGCCTCTGCTAC
speC-DnRc	<i>Xba</i> I	CAATTTCTAGAACGGGTTGTAGGCAATTTCCC
speD-UpF	<i>Xba</i> I	CTTAATCTAGAGCCCAAGGTGTTACGAAG
speD-UpRc		CGTGTGCGACGTGGGGAACCTCTC
speD-DnF		TTCCCCACGTCGCACACGAGGAAG
speD-DnRc	<i>Hind</i> III	TTAAAAGCTTAGGCGCTGTACCAGGGC
speE-UpF	<i>Hind</i> III	CTTAAAAGCTTGGCGGCCACCAGC
speE-UpRc		GGTGAAGCGGGGCCGGGATCTCCC
speE-DnF		GATCCCGGCCCGCTTCACCAAGAAG
speE-DnRc	<i>Xba</i> I	CTTAATCTAGATCGCGATGCCGTCG
spuC-UpF	<i>Xba</i> I	CTTAATCTAGAAGTGCTGCCGCTGTTC
spuC-UpRc		CTCAGGGACGTCACACCTCTTCTATTCAAG
spuC-DnF		GGTGTGACGTCCCTGAGCGGACTTTTG
spuC-DnRc	<i>Hind</i> III	CTTAAAAGCTTGTAGCCGATGCCGATGG
spuD-UpF	<i>Hind</i> III	CTTAAAAGCTTCCTGGAGAACATCCGCATC
spuD-UpRc		TCGCGGAGCGGGGTAGCTCC
spuD-DnF		ACCCCGCTCCGCGAGGAGCC
spuD-DnRc	<i>Xba</i> I	CAATTTCTAGATCTTCTTCTCCGCCTGCAC
GFPmut3-F		ATGTCTAAAGGTGAAGAATTATTC
GFPmut3-Rc		TTATTTGTACAATTCATCCATACC