Role of the transcriptional regulator ArgR in the connection between arginine metabolism and c-di-GMP signaling in *Pseudomonas putida*

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SUPPLEMENTARY MATERIAL

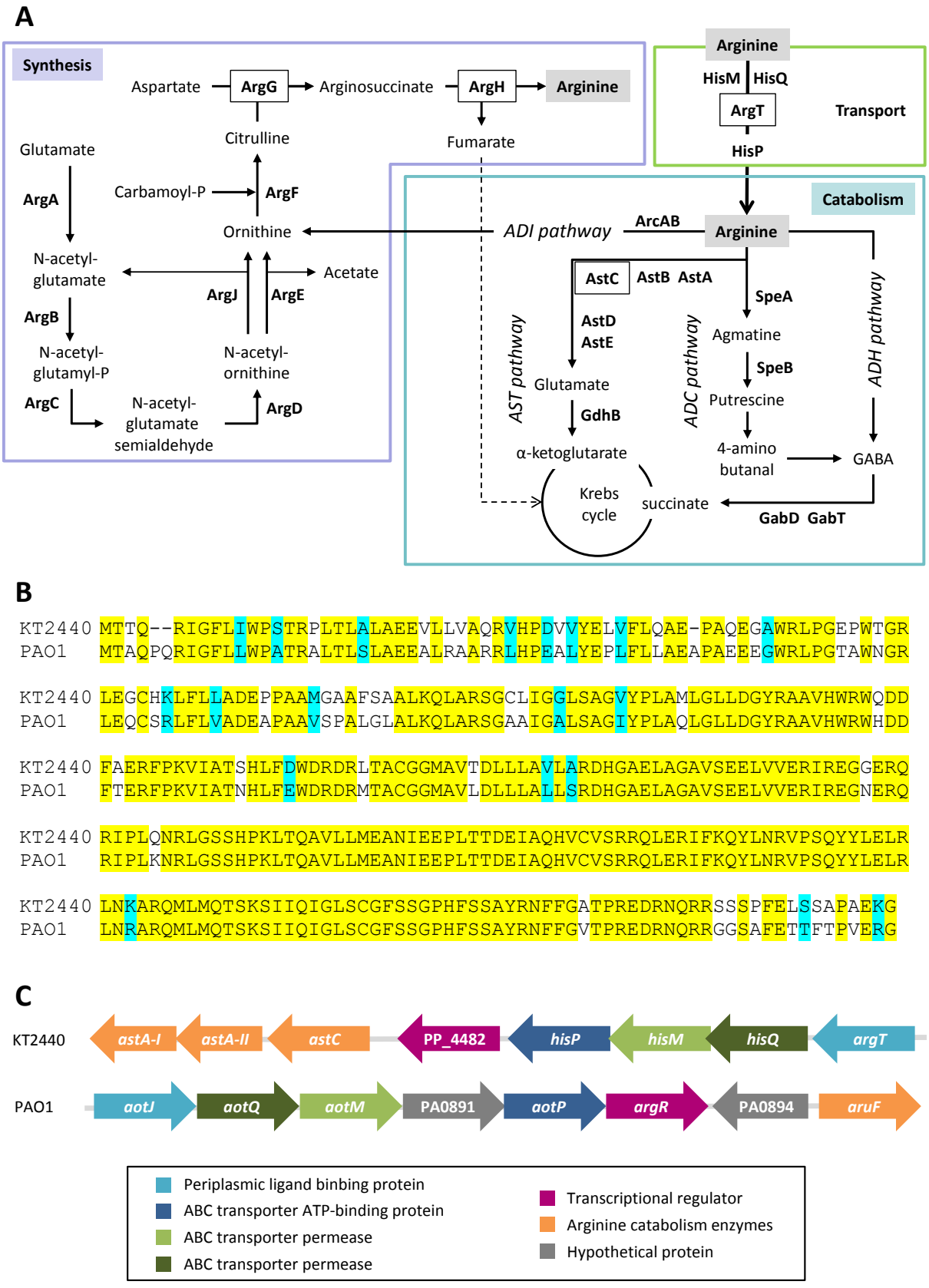
A
Synthesis
Aspartate
ArgG
Arginosuccinate
ArgH
Arginine
Citrulline
Fumarate
Glutamate
ArgA
Carbamoyl-P
ArgF
Ornithine
N-acetyl-glutamate
ArgJ
ArgE
Acetate
ArgB
N-acetyl-glutamyl-P
N-acetyl-ornithine
ArgC
N-acetyl-glutamate semialdehyde
ArgD
Arginine
HisM
HisQ
ArgT
HisP
Transport
Catabolism
ADI pathway
ArcAB
Arginine
AstC
AstB
AstA
SpeA
AST pathway
AstD
AstE
ADC pathway
Agmatine
SpeB
ADH pathway
Glutamate
GdhB
Putrescine
α-ketoglutarate
4-amino butanal
GABA
Krebs cycle
succinate
GabD
GabT
B
KT2440 MTTQ--RIGFLIWPSTRPLTLALAEEVLLVAQRVHPDVVYELVFLQAE-PAQEGAWRLPGEPWTGR
PAO1 MTAQPQRIGFLLWPATRALTLSLAEEALRAARRLHPEALYEPLFLLAEAPAEEEGWRLPGTAWNGR
KT2440 LEGCHKLFLLADEPPAAMGAAFSAALKQLARSGCLIGGLSAGVYPLAMLGLLDGYRAAVHWRWQDD
PAO1 LEQCSRLFLVADEAPAAVSPALGLALKQLARSGAAIGALSAGIYPLAQLGLLDGYRAAVHWRWHDD
KT2440 FAERFPKVIATSHLFDWDRDRLTACGGMAVTDLLLAVLARDHGAELAGAVSEELVVERIREGGERQ
PAO1 FTERFPKVIATNHLFEWDRDRMTACGGMAVLDLLLALLSRDHGAELAGAVSEELVVERIREGNERQ
KT2440 RIPLQNRLGSSHPKLTQAVLLMEANIEEPLTTDEIAQHVCVSRRQLERIFKQYLNRVPSQYYLELR
PAO1 RIPLKNRLGSSHPKLTQAVLLMEANIEEPLTTDEIAQHVCVSRRQLERIFKQYLNRVPSQYYLELR
KT2440 LNKARQMLMQTSKSIIQIGLSCGFSSGPHFSSAYRNFFGATPREDRNQRRSSSPFELSSAPAEKG
PAO1 LNRARQMLMQTSKSIIQIGLSCGFSSGPHFSSAYRNFFGVTPREDRNQRRGGSAFETTFTPVERG
C
KT2440
astA-I
astA-II
astC
PP_4482
hisP
hisM
hisQ
argT
PAO1
aotJ
aotQ
aotM
PA0891
aotP
argR
PA0894
aruF
Periplasmic ligand binbing protein
ABC transporter ATP-binding protein
ABC transporter permease
ABC transporter permease
Transcriptional regulator
Arginine catabolism enzymes
Hypothetical protein

Figure S1 (previous page)**. A**. Schematic view of arginine biosynthesis, catabolism and transport. Elements mentioned in the text connected with ArgR regulation are boxed. **B.** Sequence alignment of PP_4482 (ArgR) from *P. putida* KT2440 and ArgR from *P. aeruginosa* PAO1, done using CLUSTALW (https://www.genome.jp/tools-bin/clustalw). Identical residues in both proteins are highlighted in yellow and conservative residues in cyan. **C.** Comparison of the genomic organization of the chromosomal region containing the locus PP_4482 and putative arginine transport system in *P. putida* KT2440 (top) and the *aotJQMOP-argR* cluster in *P. aeruginosa* PAO1 (bottom). Arrows with the same color indicate gene homology. Predicted functions of the genes are indicated. Data and gene nomenclatures were obtained from the *Pseudomonas* genome database (www.pseudomonas.com; 1).

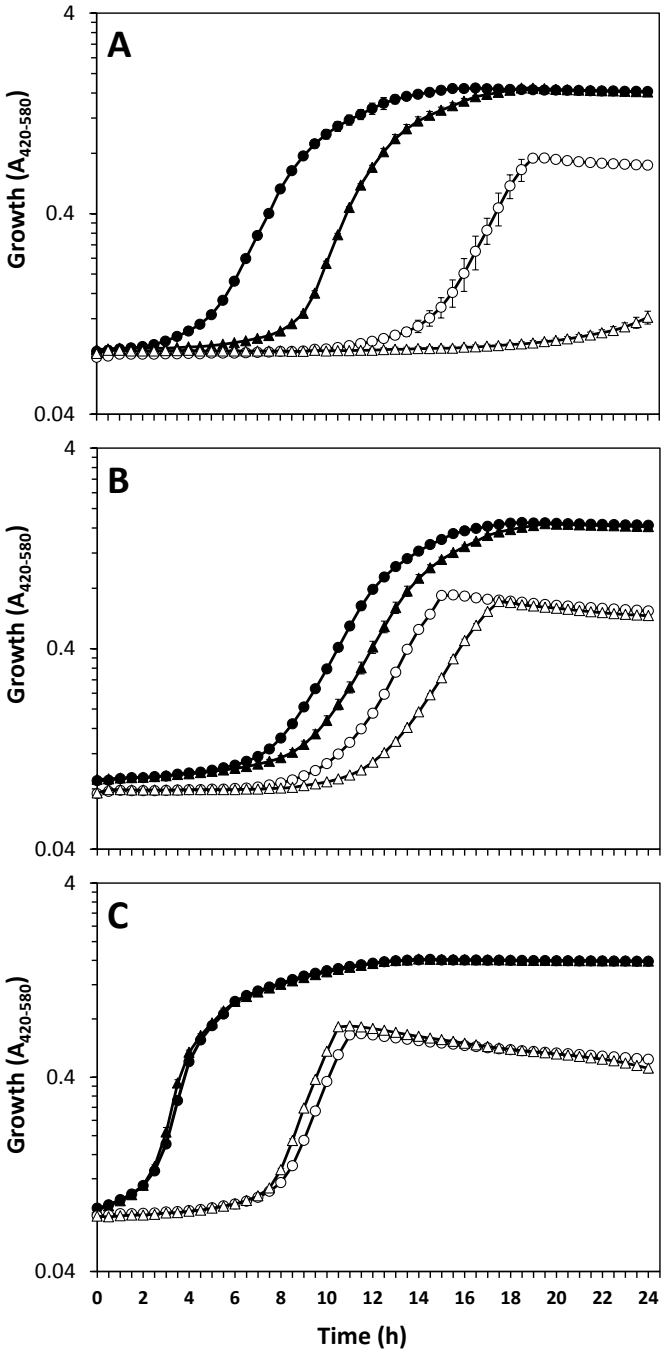

Figure S2. Growth of KT2440 (circles) and Δ*argR* (triangles) with different amino acids as carbon (open symbols) or nitrogen (filled symbols) source. L-ornithine (A), L-lysine (B) or L-histidine (C) were added (10 mM final concentration) to M9 or M8 with glucose (20 mM) media and growth at 30°C with shaking was followed using a Bioscreen™ apparatus. Absorbance in the 420-580 nm range was measured every 30 min. Averages and standard deviations of one representative experiment with three technical replicates are plotted.

gene	predicted ARG boxes	Distance to ATG (bp)
argG	**TGTCGC**TTTGTC**G**TC**A**GAAGC**TGTCGC**CCCACT**G**T**AA**	59
argT	**T**CAG**GC**GCTTCTAT**AA**GTAGT**TGTCGC**TTTGAA**G**A**AA**TATCGACTACCGGGC**TGTCG**TTAAAAT**G**CC**A**	117
astC	**TGTCGC**ATTGCC**G**A**AA**GCCT-**TGTC**AAAACTGG**G**TTTTGCGCTGTAACAAGT**TGTCGC**TTGGCC**G**C**AA**	78
spuE	**T**TT**GC**GTGTGC**G**GC**A**GCAT-**TGTCGC**TACCCA**G**GC**A**	50
argS	**TG**C**CGC**TTGTGGCCCCCAGCT**TG**C**CGC**CCAAAG**G**TTG	91

Figure S3. Potential ARG boxes identified in the upstream region of genes related to arginine metabolism in *P. putida* KT2440. Bases identical to the *P. aeruginosa* consensus sequence (2) are indicated in boldface. Highlighted bases correspond to the predicted half-sites.

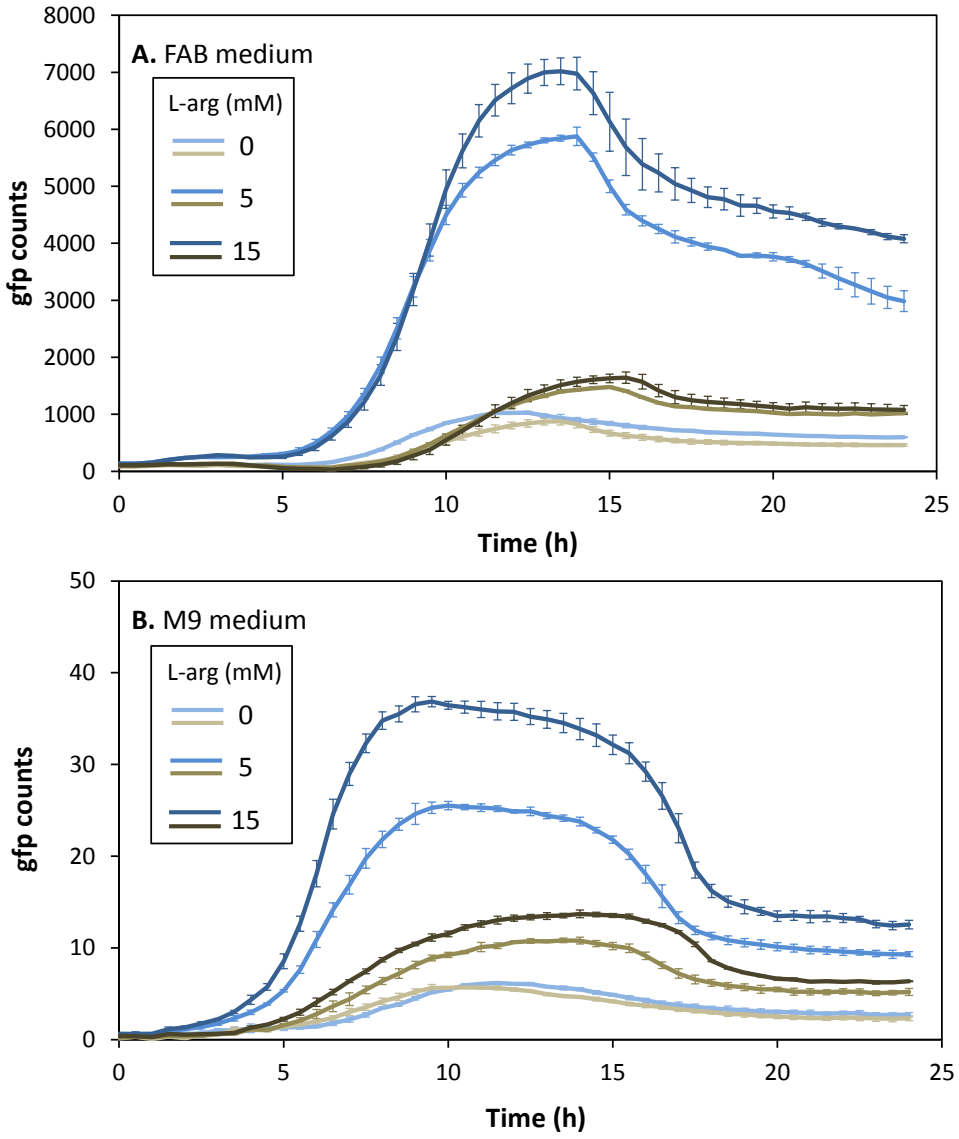

Figure S4. C-di-GMP levels in KT2440 (blue) and the Δ*argR* (olive) mutant in response to L-arginine. Strains harboring pCdrA*::gfp*^{C} were grown in modified FAB (3) **(A)** and M9 **(B)** minimal media with glucose supplied with different final concentrations of L-arginine (0, 5, and 15 mM) for 24 h. The data are presented as gfp counts which indicate fluorescence values normalized by growth (OD_{600nm}) and correspond to averages and standard deviations of one representative experiment using three experimental replicates per condition. Line color intensity indicates increasing concentrations of the amino acid. Measurements were done using TECAN Infinite 200 (A) and Varioskan Lux (B) fluorimeters.

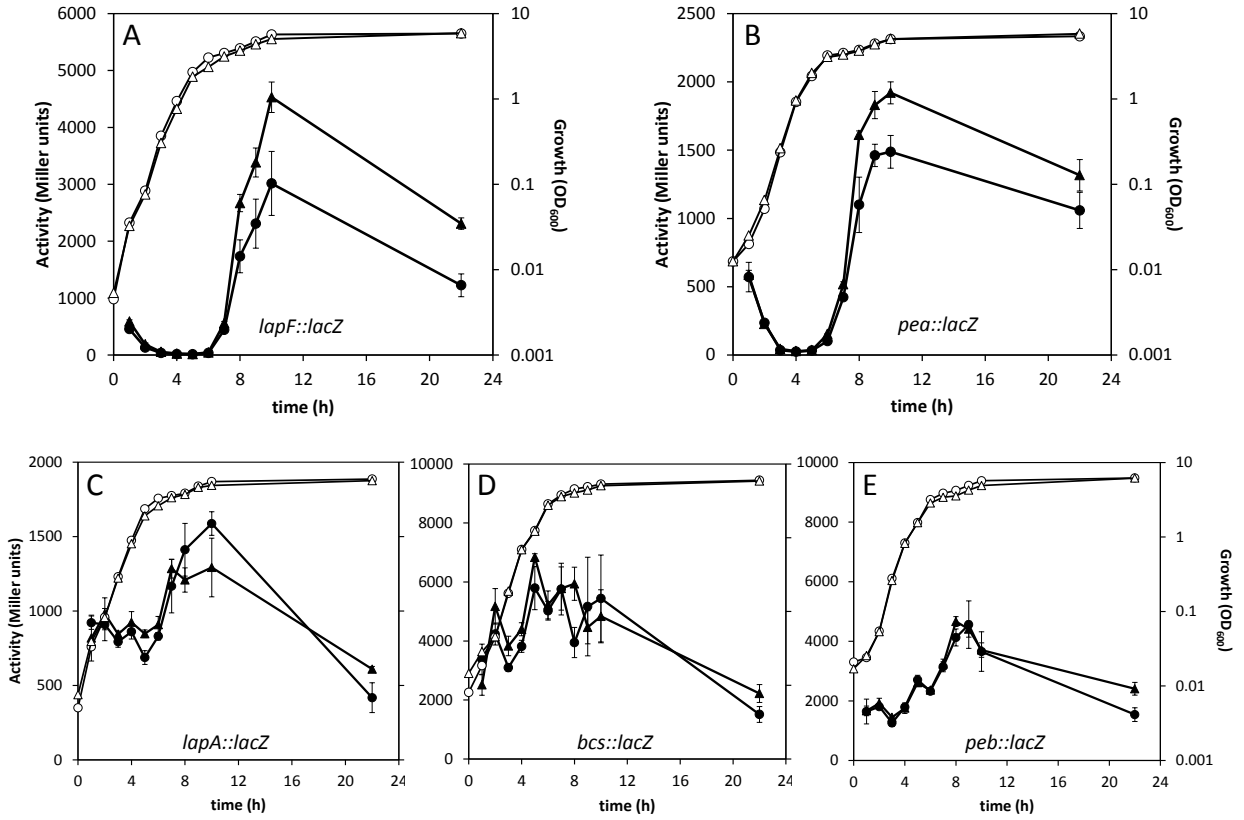

Figure S5. Expression pattern of *lapF* (A), *pea* (B), *lapA* (C), *bcs* (D) and *peb* (E) transcriptional fusions (4,5) to *lacZ* in KT2440 (circles) and the Δ*argR* mutant (triangles). Growth (open symbols) and activity (closed symbols) were followed over time in LB. Results are averages and standard deviations from 2 independent experiments with three technical replicates each.

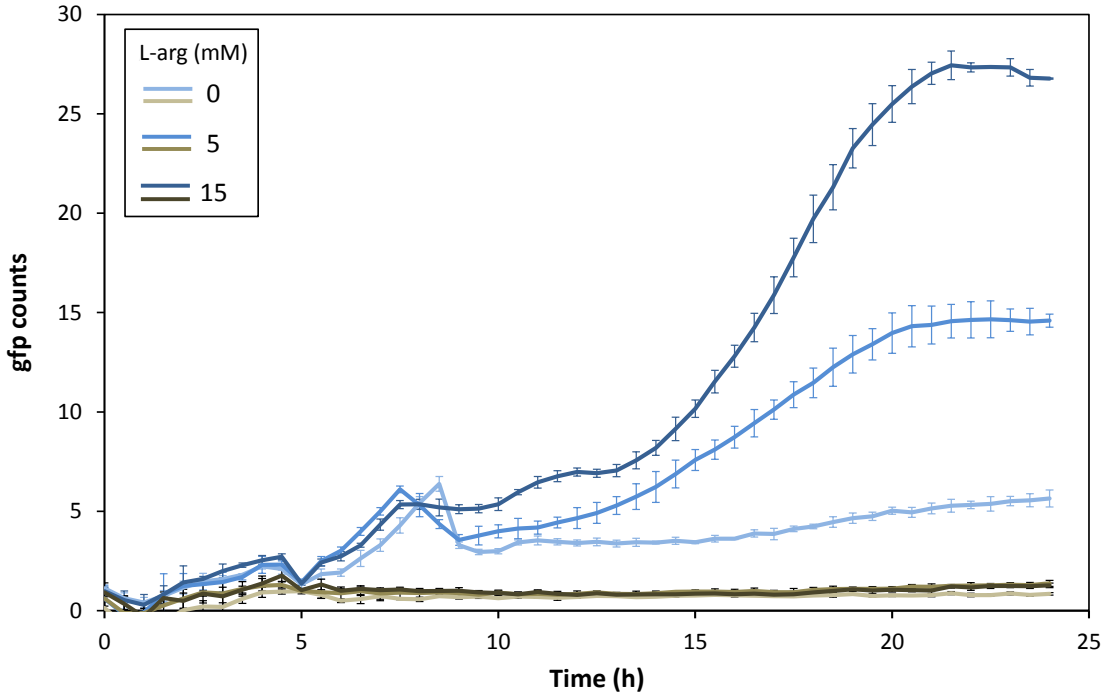

Fig S6. The change in c-di-GMP levels in response to L-arginine requires *cfcR* and *argR*. KT2440 (blue) and a Δ*argR*Δ*cfcR* mutant (olive) harboring pCdrA*::gfp*^{C} (6) were grown for 24 h in 96-well plates in diluted LB (1:3) supplied with different final concentrations of L-arginine (0, 5, and 15 mM), and fluorescence and growth (OD_{600}) were recorded every 30 min. Data are presented as gfp counts, which correspond to fluorescence values normalized by growth (OD_{600}), and are the averages and standard deviations of one representative experiment using three experimental replicates per condition. Line color intensity indicates increasing concentrations of the amino acid. Measurements were done using a Varioskan Lux fluorimeter.

REFERENCES

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