Overlap in phenotypes controlled by the cyclic di-GMP phosphodiesterase Eal in response to antibiotic exposure and DSF-mediated cell-cell signaling in *Xylella fastidiosa*

Alessandra A. de Souza[1,2,§], Michael Ionescu[1,§], Clelia Baccari[1], Aline M. da Silva[3] and Steven E. Lindow[1,†]

[1] Department of Plant and Microbial Biology, University of California, Berkeley, California 94720, USA.

[2] Centro APTA Citros Sylvio Moreira – Instituto Agronômico de Campinas, Caixa Postal 04, Cordeirópolis, SP 13490-970, Brazil .

[3] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP 05508-000, Brazil.

[§] Both authors contributed equally to this work.

[†] To whom correspondence should be addressed. Email: icelab@berkeley.edu.

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL TABLE S1

SUPPLEMENTAL FIGURES S1, S2, S3 and S4

TABLE S1: Primers used in this study

PRIMER NAME	SEQUENCE (5' → 3')	DESCRIPTION
CLONING		
kanR-F/BamHI	TTGTAGTAGGATCCTGTGTGAAATTGTTATCCG	Construction of pFXF*kan* and generating southern-blot probe
kanR-R/BamHI	TTGTCGTTGGATCCTACTGGGCTATCTGGACAAG	
PD1617–F/HindIII	TTGTAGTAAAGCTTGCTATTCCTGAAGGCGTCAATG	Construction of pFXF1, *ealXF* deletion vector
PD1617-R/XbaI	TTGTAGTATCTAGACGATAAGAGTCCAACAACGTTTC	
PD1616-F/KpnI	TTGTAGTAGGTACCGTGGTGTGGATATGGCTCAAGGC	
PD1616-R/EcoRI	TTGTAGTAGAATTCATTGCGTGAGATCGAGTGACC	
1617COMP-F/SalI	TTGTAGTAGTCGACGCCGTTACTGTGTTGCTATTCC	Construction of pFXF2, *ealXF* complementation vector
1617COMP-R/HindIII	TTGTAGTAAAGCTTCAAAAAGTCGCACACCTCG	
PD0754-F/PstI	ATTGTAGTACTGCAGTCACAAGGAGAAAAACAACCCTG	Construction of pFXF3, *clp* deletion vector
PD0754-R/XbaI	TTGTAGTATCTAGAGTAGTGTTCCCTATAGTCATATTAG	
IG-F/SacI	TTGTAGTAGAGCTCGTTGTTCTGTACGGAACGCGTTG	
IG-R/ExoRI	TTGTAGTAGAATTCACGCATTGCATTACTCATTGATAG	
EALdom(HindIII)	TTGTAGTAAAGCTTAGAGGAAGCCCATCATGCAGCGATGTGAATTCTTTGTCTGCTATC	Cloning of the EAL domain encoding region of *eal* into pBBR1MCS-2
EALdom(EcoRI)	TTGTAGTAGAATTCAGTCATCGGGATGCATACTGCGATTG	
qPCR		
rpoD	GGCTTGAGCGAGGTACAAG CGTCAACCTCAACAATGGAC	Endogenous control gene 1
rpsO	CAGGTTGCACTGTTGACGGC AAAAGACCACGGCGACTATG	Endogenous control gene 2
ealXF	AATTGGCACTGGCCACTATG CACATCATGCGAAGGATCAC	Tested genes
cgsA	TTGTTTGCTGGTGACTCTGG ATCAATATCGCGCTCCAATC	
rpfG	TGAAACCGTCTCCCGTTTAG	

	GTCGCACCTCTTCCTCAGAC	
hxfB	ACACCCACAGCTCCCACTAC TACCGGCAGCATCTACGTTG	
fimA	CGCTGCATCGGTGGCTGGAG GTAACACCTTCAACGGACTCG	
gumJ	TATCGGTTATCGGCTTGGTC CGGAACAATCACATGCAAC	
pilY1	TAACAGCGACATTTGCAAGC CTCACACCCACACCAGACAC	

SUPPLEMENTAL FIGURES

Fig. S1

Analysis of kanamycin resistance gene (*aph*(3')II) copy numbers in genomes of *X. fastidiosa* wild type, *rpfF* mutant and *eal* mutant strains by southern blot hybridization. Genomic DNA was digested with *EcoRI* to generate, in addition to various length genomic fragments, a ~4.5 Kbp genomic fragment harboring the *eal::kanR* allele. The digestion products were then separated by electrophoresis and probed with a *kanR* specific DNA probe generated by PCR using pBBR1MCS-2 as a template and the primers described in Table S1.

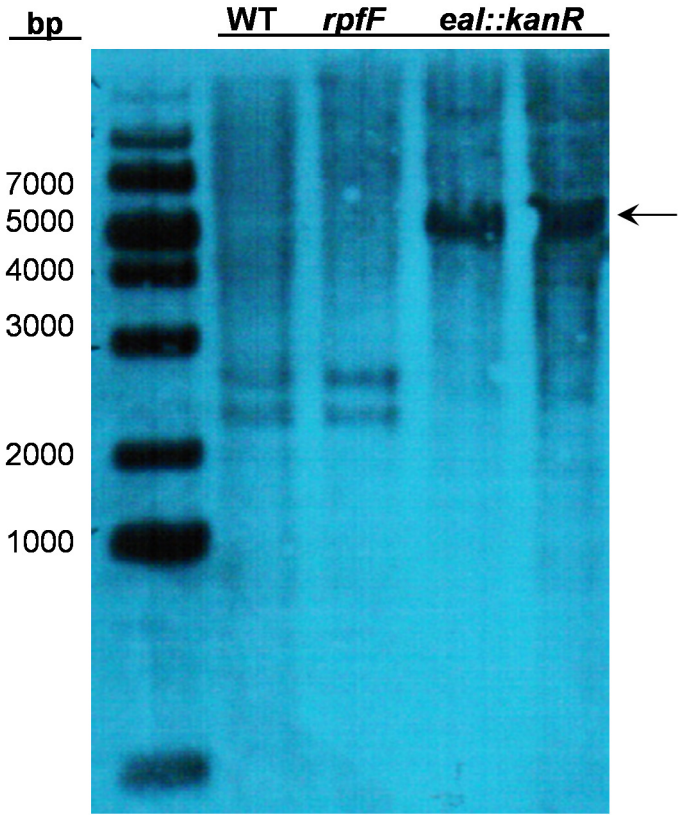


Fig. S2

Reversion of the hyper-motile phenotype of an *eal* mutant of *X. fastidiosa* by expression of *eal* from its native promoter *in-trans*. The *eal* gene was cloned into pBBR1MCS-5 and transformed into the *eal* mutant.

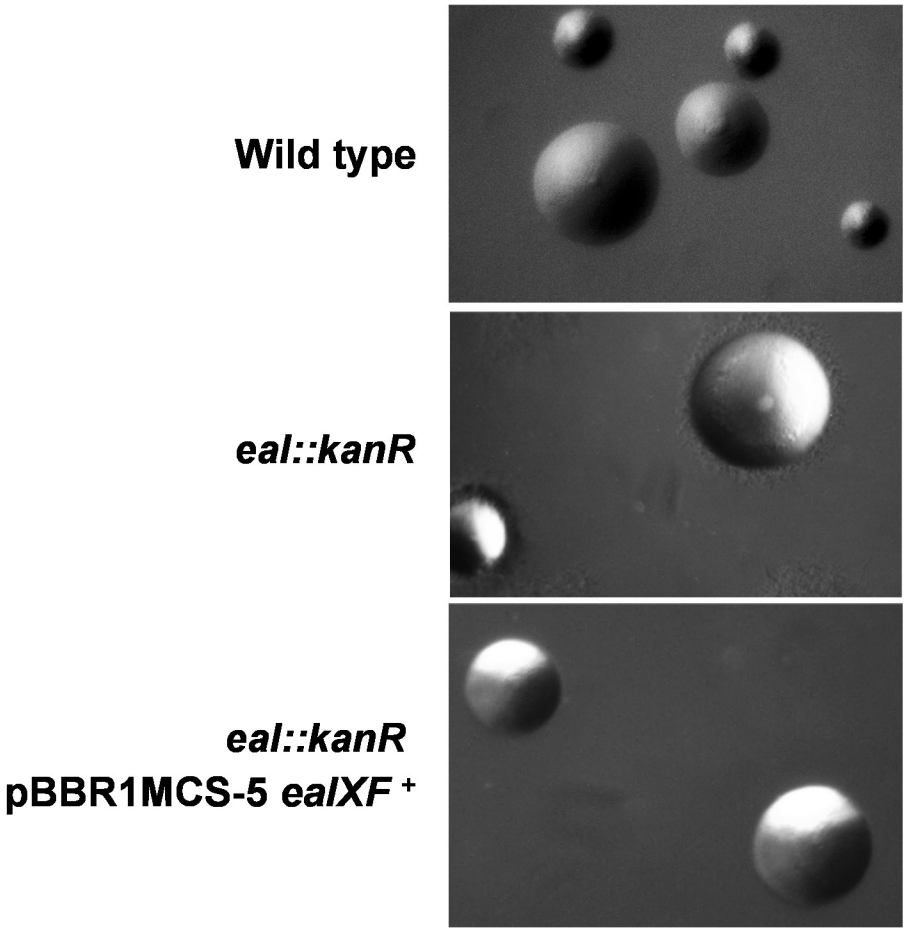
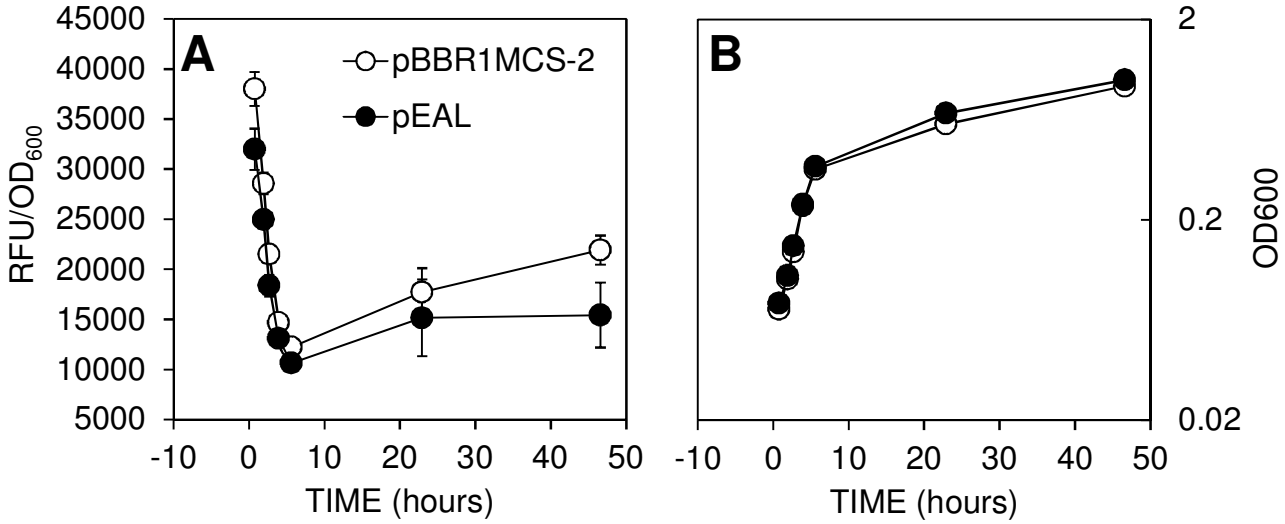


Fig. S3



Verification of *X. fastidiosa* Eal cyclic di-GMP phosphodiestrase activity. The EAL domain of Eal (see Fig. 1) was cloned into pBBR1MCS-2 (pEAL) under the control of the *lacZ* promoter and expressed in *P. aeruginosa* PAO1 Δ*pel*Δ*psl* double mutant harboring a chromosomal-based cyclic di-GMP responsive transcriptional fusion *cdrA'::gfp* (29). (A) Gfp activity. (B) Growth curve. At T=0 h, colonies grown on LB broth supplemented with kanamycin and gentamycin were suspended in ABTG+casA broth to an OD_{600} = 0.05 and incubated in a 48-well microtiter plate on 37ºC (static).

Fig. S4

Effect of tobramycin on growth and biofilm formation of the wild type *X. fastidiosa* strain. Cells were incubated for 7 days. Cell density was measured after homogenization by vigorously pipetting. Biofilm formation was measured by removing the planktonic cells and staining with crystal violet. Significant inhibition of growth occurs upon exposure to concentrations ≥ 5 µg/ml.