

Dcsbis (PA2771) from *Pseudomonas aeruginosa* is a highly active diguanylate cyclase with unique activity regulation

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Supplementary table S1

Supplementary figures S1-S3

Table S1. Strains, plasmids, and primers used in the study

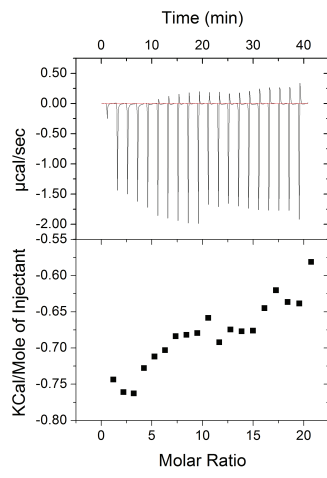
	Relevant genotype or sequence (5'-3')	Reference
<i>P. aeruginosa</i> strains		
PAO1	Wide tvpe	
<i>PA2771</i> mutant	PAO1 Δ <i>PA2771</i>	This study
<i>E. coli</i> strains		
DH5 α	Wide tvpe	
BL21(DE3)	Wide tvpe	
Plasmids		
pEX18 Gm	Suicide cloning vector, oriT+, sacB+, Gm ^r	26
pKO2771	<i>PA2771</i> knockout construct in pEX18, Gm ^r	This study
pRK2013	Helper plasmids, ColE1, oriR, Tra, Mob, Km ^r	53
pUCP18	<i>E. coli-P. aeruginosa</i> expression vector, Ap ^r , Cb ^r	54
pGL01	Expression vector, modified by pET15b, Ap ^r	This study
pPA2771	<i>PA2771</i> in pUCP18, Ap ^r , Cb ^r	This study
pPA2771C	<i>PA2771</i> (residues 173-341) in pUCP18, Ap ^r , Cb ^r	This study
pPGL1	<i>PA2771</i> in pGL01, Ap ^r	This study
pPGL173	<i>PA2771</i> (residues 173-341) in pGL01, Ap ^r	This study
Primers		
KO2771UP5	CCGGAATTCGGGACTGTCGGCGAAT	
KO2771UP3	GGGCTGGAGGACTAGCCCTGGGAGCTCACTG GATCCTGATCACGGTGGAGA	
KO2771DN5	CCACCGTGATCAGGATCCAGTGAGCTCC CAGGGCTAGTCCTCCAGCCCTGC	
KO2771DN3	CCCAAGCTTCGATAGGCGGGCGCC	
KO2771S5	CGCATTCACCCAGATTGA	
KO2771S3	CCGAACACGATCACCTGCTGGATAA	
PA2771- <i>Bam</i> HI-F	ATAGGATCCATGCTCGCCTGCCCCCTC	
PA2771- <i>Xho</i> I-3T-R	ACGTCACTCGAGTCAGACGCTGACCACCCGG TT	
PA2771-173 - <i>Bam</i> HI-F	ATA GGA TCC GAG ATC GAC CGG GAG	
PA2771-341- <i>Hind</i> III-R	CCCAAGCTTTCAGACGCTGACCACCCGGTT	

Gm^r, gentamicin resistance; Tc^r, tetracycline resistance; Ap^r, ampicillin resistance; Cb^r, carbenicillin resistance; Km^r, kanamycin resistance.

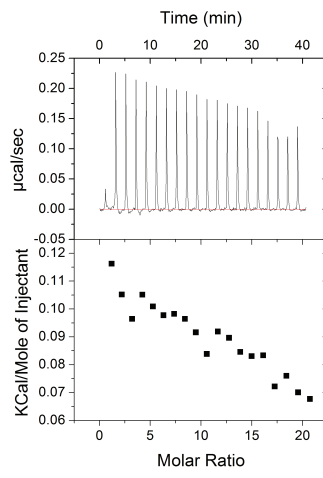
References

1. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, & Schweizer HP (1998) A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. (Translated from eng) *Gene* 212(1):77-86 (in eng).
2. Figurski DH & Helinski DR (1979) Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. (Translated from eng) *Proc Natl Acad Sci U S A* 76(4):1648-1652 (in eng).
3. Yanisch-Perron C, Vieira J, & Messing J (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. (Translated from eng) *Gene* 33(1):103-119 (in eng).

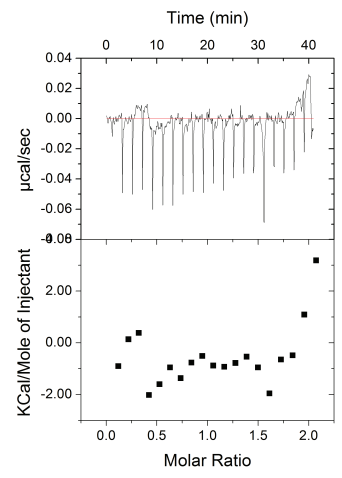
Figure S1 Binding of cAMP, cGMP and c-di-GMP to Dcsbis analyzed by ITC.



cAMP



cGMP



c-di-GMP

Figure S2 Multiple sequence alignment of the GAF domains from different proteins.

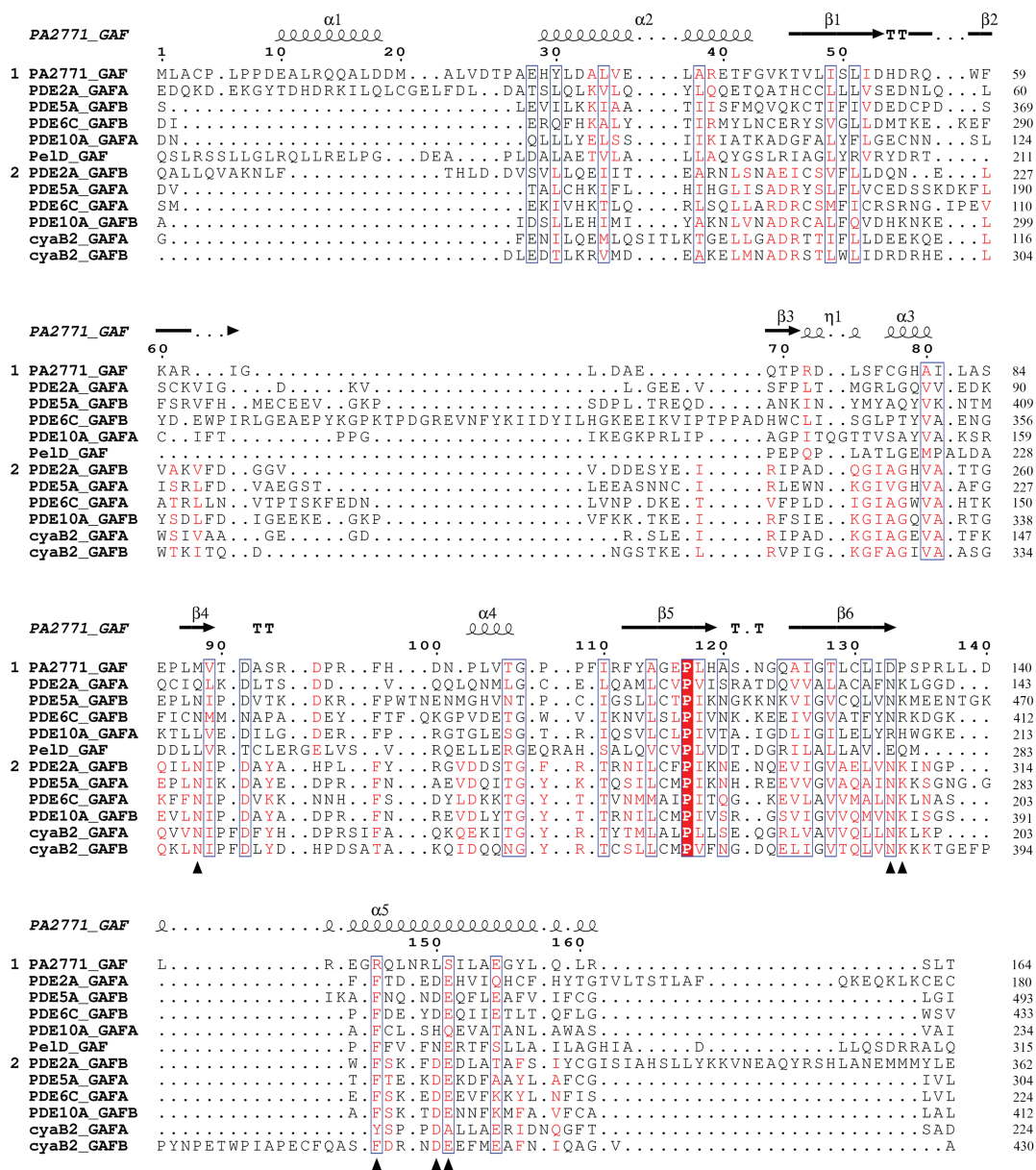


Figure S3

Sequence alignment of the protecting loop region through different *Pseudomonas* species

protecting loop

<i>Pseudomonas_aeruginosa</i> /1-341	119	HASNGQA	I	GTLC	L	IDPS	PR	137
<i>Pseudomonas_putida</i> _HB3267/1-345	119	RAGNGKA	I	GTLC	MFD	PRPR		137
<i>Pseudomonas_nitroreducens</i> /1-347	119	RAGNGQA	I	GTLC	MVD	PRPR		137
<i>Pseudomonas_mosselii</i> _SJ10/1-345	119	RAGNGKA	I	GTLC	MFD	PQPR		137
<i>Pseudomonas_thermotolerans</i> /1-342	119	RDASGM	I	LTLC	LLD	TTPR		137
<i>Pseudomonas_mendocina</i> _S5.2/1-345	119	RDVNGMP	L	GTLC	VAD	PKPR		137
<i>Pseudomonas_pseudoalcaligenes</i> _AD6/1-345	119	HDAEGVP	L	GTLC	LAD	PKAR		137
<i>Pseudomonas_sp._H1h</i> /1-321	119	QVSDGSK	L	GTLC	L	IDTK	PR	137
<i>Pseudomonas_fluorescens</i> _F113/1-324	119	GLEDGSK	L	GTLC	LLD	TRPR		137

Alignment of the protecting loop through different *Pseudomonas* species

color code:

green: >40% conservation

yellow: >50% conservation

orange: >70% conservation

red: identical