

The temperature-regulation of *Pseudomonas aeruginosa* *cmaX-cfrX-cmpX* operon reveals an intriguing molecular network involving the sigma factors AlgU and SigX

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SUPPLEMENTARY DATA

Table of contents

	Pages
Table S1: Normalized expression of the studied genes under HS or CS in the indicated genetic background relative to <i>P. aeruginosa</i> H103 at 37°C	2
Table S2: Characteristics of proteins identified by Maldi-TOF analysis	3
Table S3: Bacterial strains and plasmids used in this study	4-5
Table S4: Primer sequences of the indicated genes used for quantitative RT-qPCR reactions, transcription start sites identification and transcriptional fusions construction	6-7
Fig. S1: Flow cytometry analysis of <i>P. aeruginosa</i> H103 viability exposed or not to CS or HS	8
Fig. S2: Effect of HS and CS on <i>algU</i> expression and activity	9
Fig. S3: RpoH is not involved in the <i>cmaX</i> , <i>cfrX</i> and <i>cmpX</i> regulation in response to HS in mPAO1.	10
Figure S4: 2-DE total protein profiles of <i>P. aeruginosa</i> exposed to CS from 37°C to 4°C or not (control)	11
Fig. S5: c-di-GMP level quantification by LC/MS/MS in H103 strain exposed (square) or not to a CS (point)	12
Fig. S6: Effect of a sublethal dose of valinomycin on membrane fluidity measured by fluorescence polarization in stationary growth phase	13

Table S1: Normalized expression of the studied genes under HS or CS in the indicated genetic backgrounds relative to *P. aeruginosa* H103 at 37°C.

Target genes	Treatment	Genetic background		
		H103	$\Delta algU$	$\Delta sigX$
<i>cmaX</i>	HS	5.9 ± 1	0.9 ± 0.2	
	CS	1 ± 0.4		
<i>cfrX</i>	HS	1.7 ± 0.3	1.3 ± 0.4	
	CS	3.6 ± 0.08		1.3 ± 0.1
<i>cmpX</i>	HS	2.1 ± 0.4	1.3 ± 0.4	
	CS	3.1 ± 0.3		1. ± 0.3
<i>algU</i>	HS	2.9 ± 1.7		
	CS	1.4 ± 0.7		
<i>algD</i>	HS	19.4 ± 6.3	6 ± 0.5	
	CS	1.5 ± 0.3		
<i>rpoH</i>	HS	4 ± 1.2	1.4 ± 0.3	
	CS	1.3 ± 0.4		
<i>oprF</i>	HS	1.6 ± 0.1	0.8 ± 0.2	
	CS	1 ± 0.1		
<i>amrZ</i>	HS	0.4 ± 0.1	0.3 ± 0.03	
	CS	1 ± 0.3		
<i>sigX</i>	HS	0.8 ± 0.1	0.6 ± 0.1	
	CS	2 ± 0.6		0.9 ± 0.2
<i>accA</i>	HS	1 ± 0.1		
	CS	1.8 ± 0.2		1.4 ± 0.1
<i>accB</i>	HS			
	CS	2.7 ± 0.3		1.9 ± 0.8
PA5174	HS			
	CS	2.5 ± 0.8		1.2 ± 0.5
<i>psrA</i>	HS			
	CS	0.5 ± 0.1		0.2 ± 0.02

Table S2: Characteristics of proteins identified by Maldi-TOF analysis.

Spot number	RefSeq	PA number	Protein name	Theoretical MW(kDa)/pI	method	Protein Score	Number of Matched peptides	Sequence coverage (%)
1	NP_251641.1	PA2951	EtfA	31.4/4.75	MS/MS	54	1	5
2	NP_259243.1	PA0552	Pgk	40.4/5.06	MS/MS	123	2	5
3	NP_251641.1	PA2953	EtfD	59.9/5.6	MS/MS	42	1	1
4	NP_253858.1	PA5171	ArcA	46.4/5.5	MS	167	12	32
5	NP_249556.1	PA0865	Hpd	39.9/4.89	MS	133	12	29
6	NP_252329.1	PA3639	AccA	34.9/5.15	MS	84	7	26
7	NP_252345	PA3655	Tsf	30.7/4.95	MS	135	9	25
8	NP_252955	PA4265	TufA	43.3/5.03	MS	133	9	29
9	NP_253428.1	PA4740	Pnp	75.4/4.81	MS	112	9	17

Table S3: Bacterial strains and plasmids used in this study.

Cb^r, carbenicillin resistance ; Gm^r, gentamycin resistance ; Tc^r, tetracycline resistance.

Strains/plasmids	Relevant characteristic(s)	Sources
Strains		
<i>E. coli</i>		
JM109	Cloning host	Promega
S17.1	Conjugation strain	Simon et al., 1983
<i>Pseudomonas aeruginosa</i>		
H103	PAO1 derivative	Hancock, R. E. & Carey, 1979
mPAO1	PAO1 derivative	Jacobs, M. A. et al., 2003
$\Delta sigX$ (PAOSX)	PAO1 H103 $\Delta sigX$	Bouffartigues et al., 2012
$\Delta rpoH$	Transposon mutant <i>rpoH::IS;phoA/hah-Tc^r</i>	Jacobs, M. A. et al., 2003
$\Delta algU$	PAO1 H103 $\Delta algU$	This study
Plasmids		
pAB133	Vector for transcriptional fusion analyzes. Gm ^r	Bazire et al., 2005
pABcmaXL	pAB-PcmaX-luxCDABE. Gm ^r	This study
pABcfrXL	pAB-PcfrX-luxCDABE. Gm ^r	This study
pABcmpXL	pAB-PcmpX-luxCDABE. Gm ^r	This study
pEXUGL	pEXalgU with <i>lox-aacC1-lox</i> cassette. Ap ^r , Gm ^r	Bazire et al., 2010
pCM157	<i>cre</i> expression vector, Tc ^r	Quenée et al., 2005

References for Table S3

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- Hancock, R. E. & Carey, A. M. Outer membrane of *Pseudomonas aeruginosa*: heat- 2-mercaptoethanol-modifiable proteins. *J. Bacteriol.* **140**, 902–910 (1979).
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- Quénéée, L., Lamotte, D. & Polack, B. Combined sacB-based negative selection and cre-lox antibiotic marker recycling for efficient gene deletion in *pseudomonas aeruginosa*. *BioTechniques* **38**, 63–67 (2005).
- Bazire, A. *et al.* The sigma factor AlgU plays a key role in formation of robust biofilms by nonmuroid *Pseudomonas aeruginosa*. *J. Bacteriol.* **192**, 3001–3010 (2010).
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Table S4: Primer sequences of the indicated genes used for quantitative RT-qPCR reactions, transcription start sites identification and transcriptional fusions construction.

PA number	Gene name	Primer name	Sequence (5' - 3')
RT-qPCR			
PA1773	<i>cmaX</i>	PA1773	AAAGGGCCGAAGACAGCAT
		PA1773	CGCGATCGGTCAGGTAGTG
PA1774	<i>cfrX</i>	PA1774	CTGCGGGACCTCGTCAAG
		PA1774	GCCGACCTGGCGATTG
PA1775	<i>cmpX</i>	FPA1775	GGCAGATCATTGCAGGAATCTAC
		RPA1775	TCTCTCAATAGTGCCTTCAACGT
PA1609	<i>fabB</i>	F1609	CCTCGGCAATGACAAAGACA
		R1609	GTAGGACGGATTGAAGCGAATG
PA0762	<i>algU</i>	FalgU	TACCTGGCTGTATCGGATCG
		RalgU	GAAGAACTCCGCATCCTCTG
PA3540	<i>algD</i>	FPA3540	GGGCTATGTGGTGCAGTATG
		RPA3540	GCGACTTGCCCTGGTTGAT
PA	<i>rpoH</i>	FrpoH	CGCAAACCTGGAAGCCTA
		RrpoH	GAACAACGAAGCGCAGGT
PA1777	<i>oprF</i>	FPA1777	GCGTACAGCTGGACATGAAG
		RPA1777	TTCATGAAGTCAGCCAGGTTCTT
PA3385	<i>amrZ</i>	FamrZ	CGTGAGCAGATCGCAGAA
		RamrZ	GCTCGTGCAGGCTGAGTT
PA1776	<i>sigX</i>	FPA1776	AATTGATGCGGCGTTACCA
		RPA1776	CCAGGTAGCGGGCACAGA
PA3639	<i>accA</i>	FaccA	TCTTCGGCAATCTGACCAGTT
		RaccA	GTAGCCGATGTAGTCGAGGGTA
PA4847	<i>accB</i>	FaccB	AAGCCATGAAGATGATGAACC
		RaccB	CGTTCTCCACCAGGATCGA
PA5174	<i>fabY</i>	FPA5174	AGGGCGACCTGGAGATCAT
		RPA5174	GCGCGTCCTTCTTGTATACCA
PA3006	<i>psrA</i>	FpsrA	CGATTTCCGGCGTGAACAATT
		RpsrA	CGAAGAACGGCACCATCAG
PA0668.1	<i>16SrRNA</i>	F16SRNA	AACCTGGGAACCTGCATCCAA
		R16SRNA	CTTCGCCACTGGTGTTCCTT
RACE-PCR			
PA1775	<i>cmpX</i>	ASP1cmpX	CCTTCAACGTCACCCACTTT
		ASP2cmpX	AACACCTTGGGCAGGTACAG

PA1774	<i>cfrX</i>	AS1 <i>cfrX</i>	GCAGAAGATGCGTTTTTCG
		ASP2 <i>cfrX</i>	GCGGAAATACGGGAAACC
PA1773	<i>cmaX</i>	ASP1 <i>cmaX</i>	CAGGTAGTGCGCGAGGTAGT
		ASP2 <i>cmaX</i>	CGGATTCAGGTTCACTCCAC

Transcriptional fusions

**PA1773	** <i>cmaX</i>	FC <i>cmaX</i>	*taataagagctcGTTTCGTGTATGCCGACAACA
		RC <i>cmaX</i>	*taataactagttGCGCTTCCCTTGGTAAAAC
**PA1774	** <i>cfrX</i>	FCFR <i>X</i>	*taataagagctcGAGTTGATACGGGAGCGCAT
		RCFR <i>X</i>	*taataactagttCATGTCTGTACGAGGGAGTG
**PA1775	** <i>cmpX</i>	FC <i>cmpX</i>	*taataagagctcCTGCGGGACCTGCTCAAG
		RC <i>cmpX</i>	*taataactagttCACTCAGCCTTGTCAGC

* Nucleotides not in the chromosomal sequence are indicated in lower case.

** Gene localized downstream the PCR-amplified DNA fragment

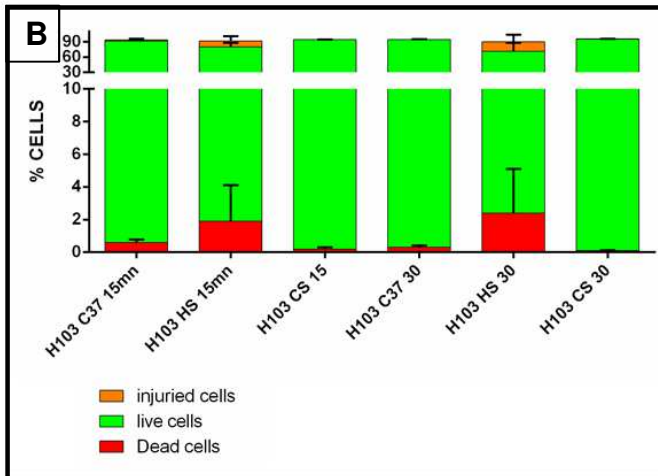
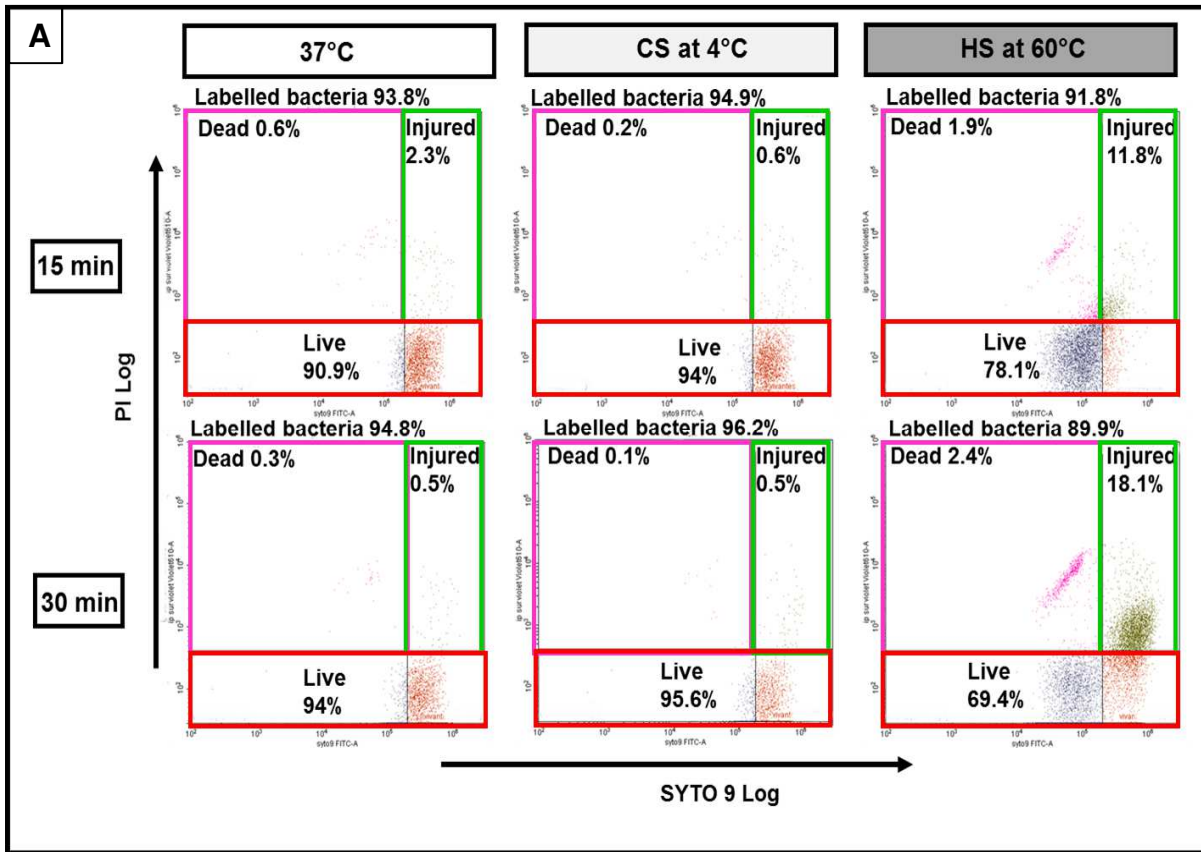


Fig. S1: Flow cytometry analysis of *P. aeruginosa* H103 viability exposed or not to CS or HS.

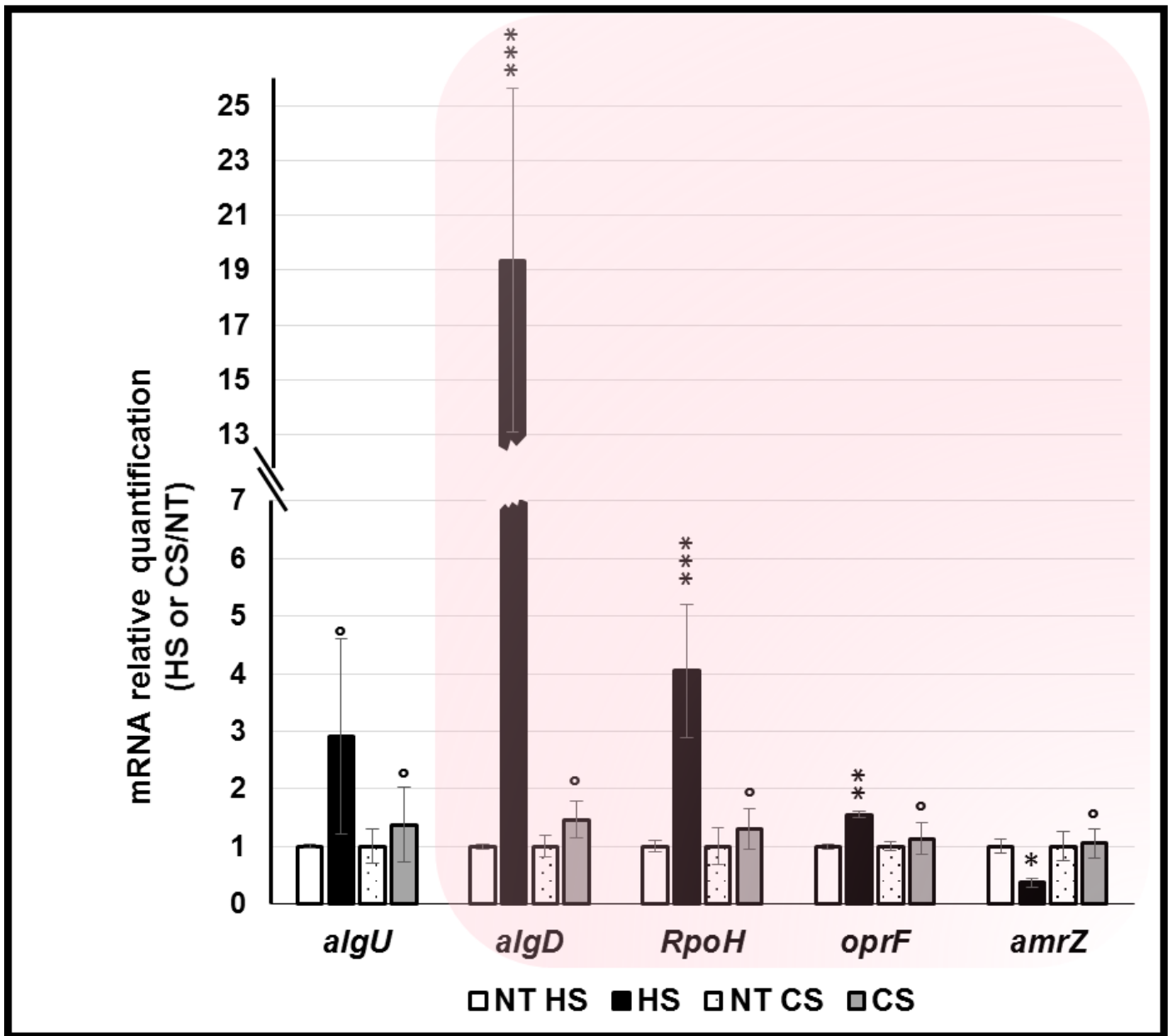


Fig. S2: Effect of HS and CS on *algU* expression and activity.

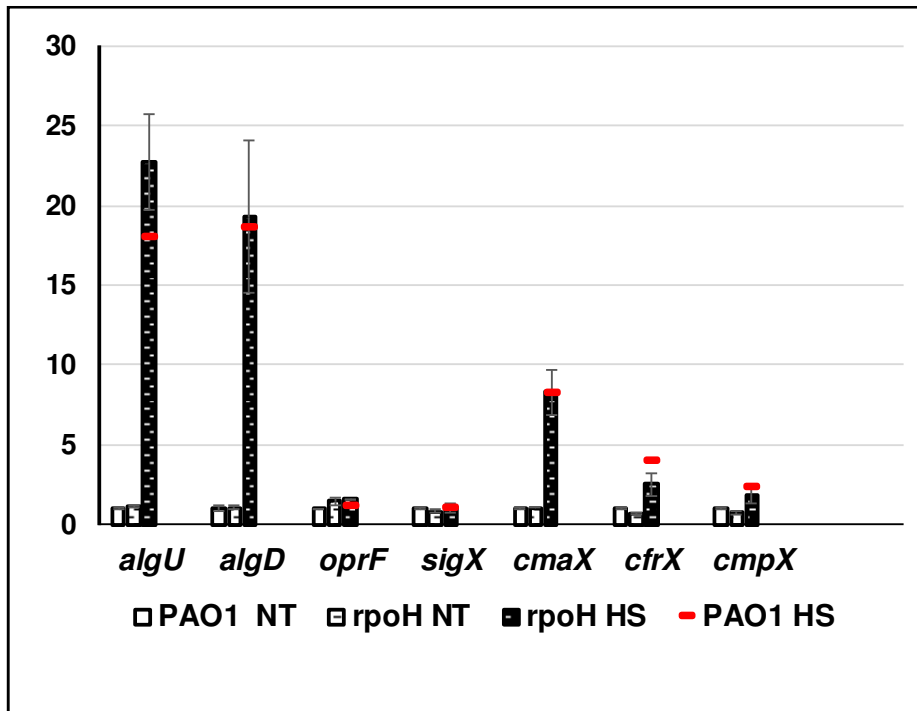


Fig. S3: RpoH is not involved in the *cmaX*, *cfrX* and *cmpX* regulation in response to HS in PAO1.

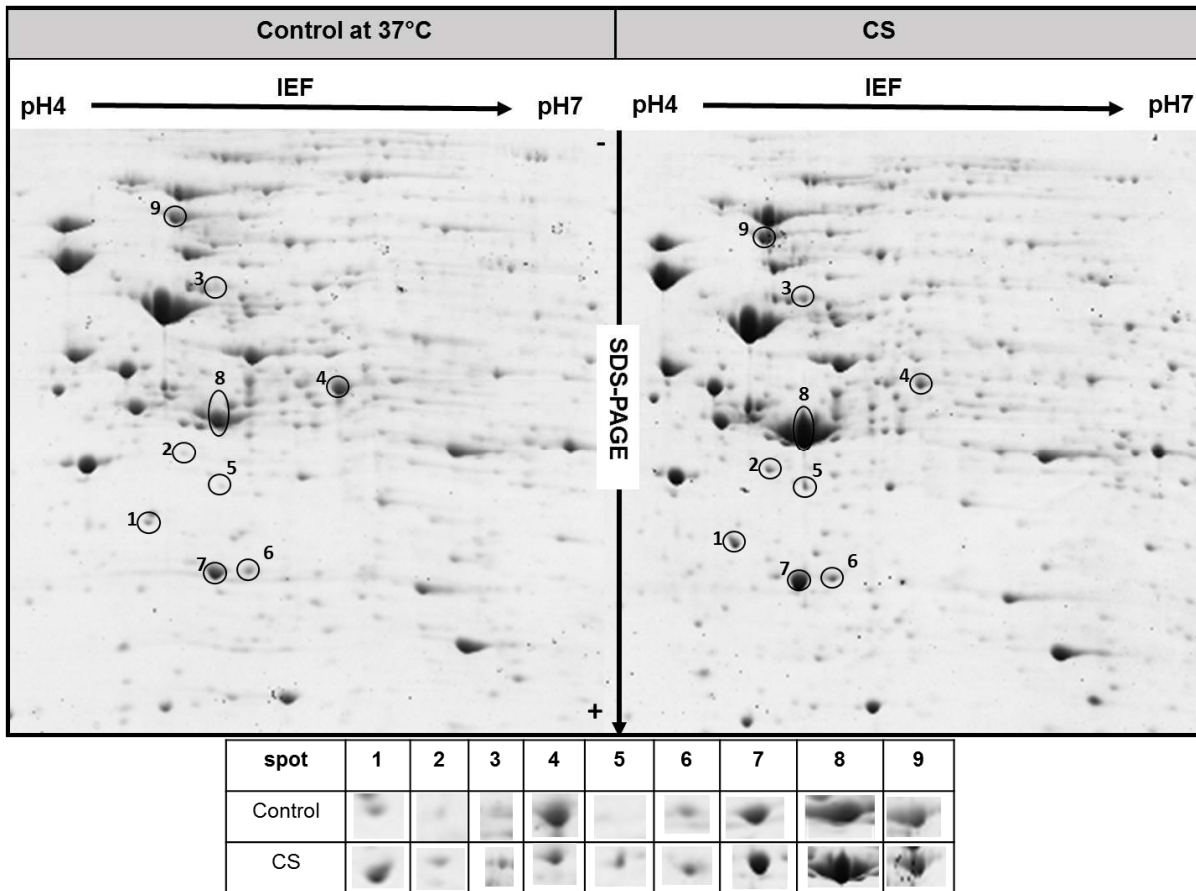


Fig. S4: 2-DE total protein profiles of *P. aeruginosa* exposed to CS from 37°C to 4°C or not (control).

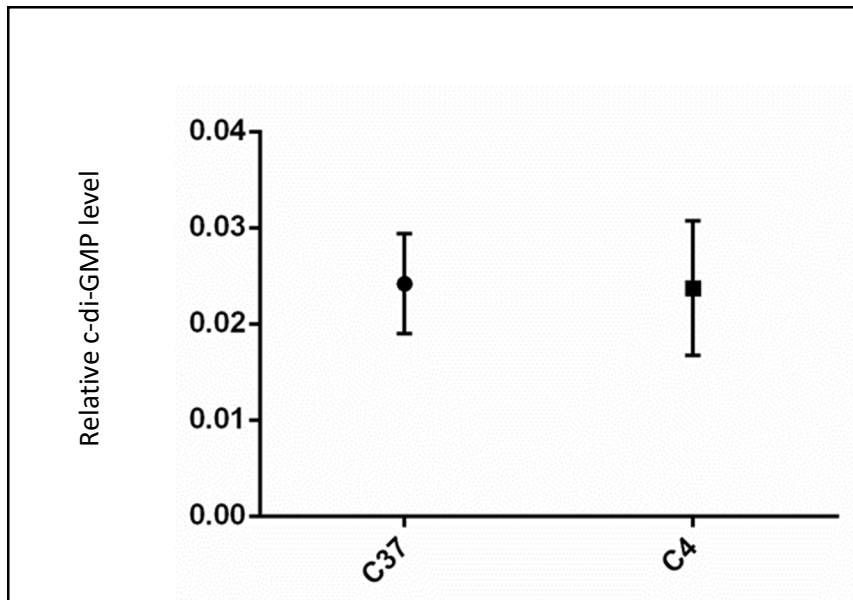


Fig. S5: c-di-GMP level quantification by LC-MS/MS in H103 strain exposed (square) or not to a CS (point).

References for Supplementary Fig. S5

Strehmel, J. *et al.* Sensor kinase PA4398 modulates swarming motility and biofilm formation in *Pseudomonas aeruginosa* PA14. *Appl Environ Microbiol.* 81(4):1274-85. (2015).

Bouffartigues, E. *et al.* The absence of the *Pseudomonas aeruginosa* OprF protein leads to increased biofilm formation through variation in c-di-GMP level. *Front Microbiol.*; 6: 630. (2015).

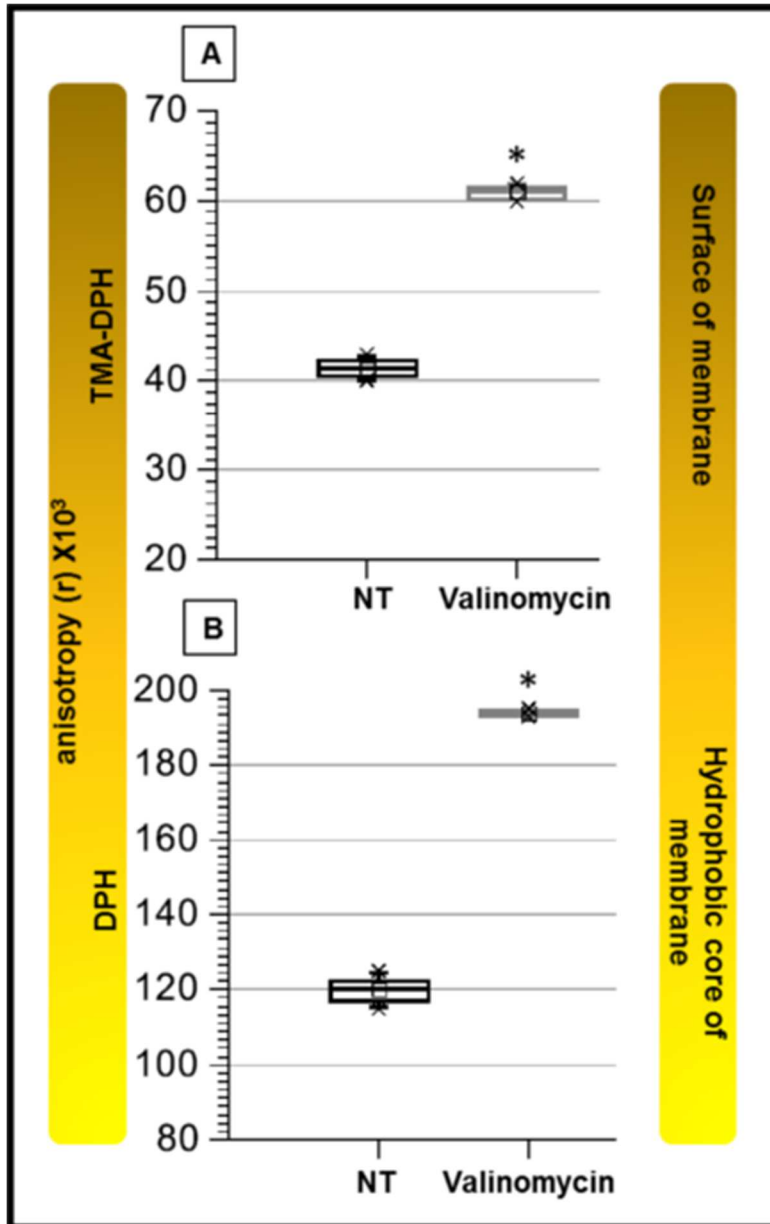


Fig. S6: Effect of a sublethal dose of valinomycin on membrane fluidity measured by fluorescence polarization in stationary growth phase.