Supplemental materials for

"Coordinated c-di-GMP repression of *Salmonella* motility through YcgR and cellulose" by Zorraquino V. et al.

Table S1. Strains and plasmids used in this study

Table S2. Oligonucleotides used in this study

Figure S1. Characterization of the deletion present in ΔXII strain

Figure S2. Phenotypic analysis of new ΔXII strain

Supplemental references

Table S1 Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	MIC[a]	Reference or source
Strains			
S. Enteritidis			
3934	Wild-type clinical isolate	54	(8)
Δ*ycgR*	3934 Δ*ycgR*::Tc^R	3068	This study
Δ*yhjH*	3934 Δ*yhjH*::Km^R	1346	This study
Δ*yhjH* Δ*ycgR*	3934 Δ*yhjH*::Km^R Δ*ycgR*::Tc^R	1360	This study
Δ*bcsA*	3934 Δ*bcsA*::Cm^R	3702	This study
Δ*ycgR* Δ*bcsA*	3934 Δ*ycgR*::Tc^R Δ*bcsA*::Cm^R	2058	This study
Δ*yhjH* Δ*bcsA*	3934 Δ*yhjH*::Km^{R}Δ*bcsA*::Cm^R	2507	This study
Δ*yhjH* Δ*ycgR* Δ*bcsA*	3934 Δ*yhjH*::Km^R Δ*ycgR*::Tc^R Δ*bcsA*::Cm^R	2650	This study
ΔXII	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316*	1324	This study
ΔXII Δ*ycgR*	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* Δ*ycgR*::Tc^R	2051	This study
ΔXII P$_{sen4316}$::*hmsT*	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* P$_{sen4316}$::*hmsT*	1403	This study
ΔXII P$_{sen4316}$::*hmsT*-GS	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* P$_{sen4316}$::*hmsT*_E288G/E289S	2164	This study
ΔXII P$_{sen4316}$::*hmsT*-3xFlag	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* P$_{sen4316}$::*hmsT*::3xFlag	2166	This study
ΔXII + *sen4316*-3xFlag	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* *sen4316*::3xFlag-Km^R	3913	This study
ΔXII Δ*ycgR* P$_{sen4316}$::*hmsT*	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* Δ*ycgR*::Tc^R P$_{sen4316}$::*hmsT*	1405	This study
CheY_D57A	3934 *cheY*_D57A	1256	This study

Strain	Genotype	No.	Source
CheY_D13K/Y106W	3934 *cheY_D13K/Y106W*	1248	This study
ΔXII Δ*ycgR* Δ*bcsA*	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* Δ*ycgR*::Tc^R Δ*bcsA*::Cm^R	2053	This study
ΔXII Δ*ycgR* Δ*bcsA* $P_{sen4316}$::*hmsT*	3934 3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* Δ*ycgR*::Tc^R Δ*bcsA*::Cm^R $P_{sen4316}$::*hmsT*	2056	This study
ΔXII Δ*bcsA*	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* Δ*bcsA*::Cm^R	2052	This study
ΔXII Δ*bcsA* $P_{sen4316}$::*hmsT*	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* Δ*bcsA*::Cm^R $P_{sen4316}$::*hmsT*	2055	This study
BcsA RxxxD	3934 *bcsA_R700D*	4040	This study
Δ*ycgR* BcsA RxxxD	3934 Δ*ycgR*::Tc^R *bcsA_R700D*	4049	This study
ΔXII BcsA RxxxD	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* *bcsA_R700D*	4043	This study
ΔXII Δ*ycgR* BcsA RxxxD	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* Δ*ycgR*::Tc^R *bcsA_R700D*	4045	This study
ΔXII BcsA RxxxD $P_{sen4316}$::*hmsT*	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* *bcsA_R700D* $P_{sen4316}$::*hmsT*	4044	This study
ΔXII Δ*ycgR* BcsA RxxxD $P_{sen4316}$::*hmsT*	3934 Δ*adrA* Δ*sen1023* Δ*yeaJ* Δ*yciR* Δ*yegE* Δ*yfiN* Δ*yhdA* Δ*sen3222* Δ*yhjK* Δ*sen2484* Δ*yfeA* Δ*sen4316* Δ*ycgR*::Tc^R *bcsA_R700D* $P_{sen4316}$::*hmsT*	4046	This study

S. Typhimurium

14028	Wild-Type	685	American Type Culture Collection
14028 Δ*yhjH*	14028 Δ*yhjH*::Km^R	4288	This study
14028 Δ*yhjH* Δ*bcsA*	14028 Δ*yhjH*::Km^R Δ*bcsA*::Cm^R	4290	This study
14028 Δ*yhjH* Δ*ycgR*	14028 Δ*yhjH*::Km^R Δ*ycgR*::Tc^R	4291	This study
14028 Δ*yhjH* Δ*ycgR* Δ*bcsA*	14028 Δ*yhjH*::Km^R Δ*ycgR*::Tc^R Δ*bcsA*::Cm^R	4292	This study
UMR1	ATCC 14028-1s Nal^R	3815	(7)

UMR1 Δ*yhjH*	UMR1 Δ*yhjH*::Km^R	4294	This study
UMR1 Δ*yhjH* Δ*bcsA*	UMR1 Δ*yhjH*::Km^R Δ*bcsA*::Cm^R	4296	This study
UMR1 Δ*yhjH* Δ*ycgR*	UMR1 Δ*yhjH*::Km^R Δ*ycgR*::Tc^R	4297	This study
UMR1 Δ*yhjH* Δ*ycgR* Δ*bcsA*	UMR1 Δ*yhjH*::Km^R Δ*ycgR*::Tc^R Δ*bcsA*::Cm^R	4298	This study
TT3699 *ara652*::Tn10	Used as template for tetracycline cassette resistance amplification	2920	Gift form G. Casadesús
SV4406 *rcsB*::MudQ	Used as template for chloramphenicol cassette resistance amplification	748	Gift form F. García del Portillo
E. coli			
MC4100 *ybeW*::Km	Used as template for kanamycin cassette resistance amplification	3015	Gift form J. M. Ghigo
XL1Blue	*recA1 endA1 gyrA96 thi*-1 *hsd*R17 *supE*44 *rel*A1 *lac* [F´*proAB lacI*^{q} Z D*M15* Tn10(Tc^R)]	797	Stratagene

Plasmids

pKOBEGA	Amp^R, vector for recombination experiments	(3)
pKO3Blue	Cm^R, derivative of pKO3 carrying the pMAD *lacZ* gene under control of the P*clpB* promoter. Vector used to generate point mutation in CheY and to construct ΔXII and ΔXII derivative strains	(9)
pBR328	Cloning vector Amp^R, Km^R, Cm^R	(2)
pBR328::*stm1987*	pBR328 containing *stm1987* from *S.* Typhimurium 14028	(4)
pBR328::*stm1987*-GS	pBR328::*stm1987*_E479G/E480S	(4)
pGFP	pMPMA3ΔPlac containing *gfp*[LVA] without a promoter	(5)
pP*fliC*GFP	pMPMA3ΔPlac containing *gfp*[LVA] downstream the *fliC* promoter	(5)
pUA1108	pGEX 4T-1 derivative plasmid carrying the P*tac* promoter and the *lacI*^{q} gene; Amp^R	(6)
pUA1108::*bcsZ*	pUA1108 containing the *sen3440* gene under the control of the P*tac* promoter	This study
pRW99	pKD46 with I-SceI endonuclease under control of tetracycline-inducible promoter (P$_{tetA}$), temperature-sensitive, orientation 2 (5'->3': I-SceI-tet^R), Amp^R	(1)

pRW100	pKD3 with I-SceI recognition site, Cm^R. Use for chloramphenicol cassette amplification in rapid mutagenesis.	(1)

a. Number of each strain in the culture collection of the Laboratory of Microbial Biofilms, Instituto de Agrobiotecnología (Idab).

Table S2 Oligonucleotides used in this study

Primer	Sequence (5′–3′)
Gene deletion	
yhjH Km Fw	gaacgccgataaccttttgacgagtccggacagtcacactcccattaacaggacaactgagaaagcca cgttgtgtctcaa[a]
yhjH Km Rv	agacgaaaaaggaggatgaccgcgcctcgtaataccacgtattacgggaacagtctggcggcgctg aggtctgcctcgtg[a]
ycgR Tet Fw	gtgagtggttacaatgagcagttcctgaaaaaaaatccattagcgatattaggcgtgctacgctgttaat cactttactt[b]
ycgR Tet Rv	ttattctcgcactttattcgctctttctcgggcctcgcgctcaagcgaaaaaataatccgggttatcaaga gggtcatta[b]
bcsA Clo Fw	tcattgttgagcctgagccataacccgatccgacggctgtatcgccgcttgccgctccgggtgtaggct ggagctgcttc[c]
bcsA Clo Rv	ccctctggctggtgatgatctcgtcgactaaagaacgtcatgaaccgcgctggcggtcatcatatgaat atcctccttag[c]
Aa substitutions	
cheY-E	ggctcgagaaactctgtcaaatcagcg
cheY-F	ggctcgaggctggtatggcccggg
cheY Fw	ggctcgagaaactctgtcaaatcagcg
cheY Rv	ggctcgaggctggtatggcccggg
cheY Mut1 Fw	cttaaattttttggttgtggataaattttcgaccatgcgtcgt
cheY Mut1 Rv	acgacgcatggtcgaaaatttatccacaaccaaaaatttaag
cheY Mut1 Comp	acgacgcatggtcgaaaattt
cheY Mut2 Fw	gctggcgccagcggttgggtcgtaaaaccgttcacc
cheY Mut2 Rv	ggtgaacggttttacgacccaaccgctggcgccagc
cheY Mut2 Comp	gctggcgccagcggttgg
cheY Mut3 Fw	gctttggttttattatctccgcctggaacatgccgaacatg
cheY Mut3 Rv	catgttcggcatgttccaggcggagataataaaaccaaagc
cheY Mut3 Comp	catgttcggcatgttccaggcgg
PilZ Clo Fw	attctcggcggcgcggttgcggtttcggtagagagtaaacaggtcaggcgcgcgcatcggcgccta cgccccgccctgc[c]

PilZ Clo Rv	ggtacaggagaacaaatgtccatcttcgcgggcgatggcccccggcatggcaatctcgac<u>ctagact</u> <u>atattaccctgtt</u>[c]
PilZ Dimer Fw	tgcggtttcggtagagagtaaacaggtcaggcgcgcgcatgacgtcgagattgccatgccgggggc catcgcccgcgaagatg
PilZ Dimer Rv	catcttcgcgggcgatggcccccggcatggcaatctcgacgtcatgcgcgcgcctgacctgtttactc tctaccgaaaccgca
bcsA-A	agatctgggtcttctataacctgg
bcsA-B	ggatccaacccgatccgacggc
MuL	cgaataatccaatgtcctcc
pcm Fw	acgtgtacaggctcttct
sen2747 Rv	ttgagttcggaagcatcc

BcsZ overexpression

bcsZ NdeI Fw	gagagagg<u>catat</u>gatgactatgctgcg[d]
bcsZ NotI Rv	ctctctcc<u>gcggccgc</u>ttaacgtgaacttacgcatt[e]

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a. Priming sequence for the Km resistance gene underlined.
b. Priming sequence for the Tet resistance gene underlined.
c. Priming sequence for the Clo resistance gene underlined.
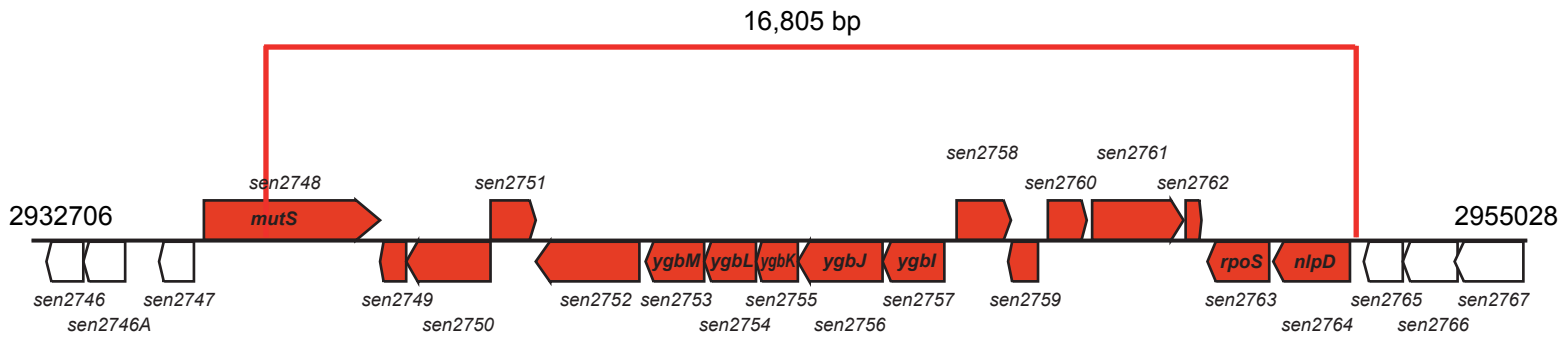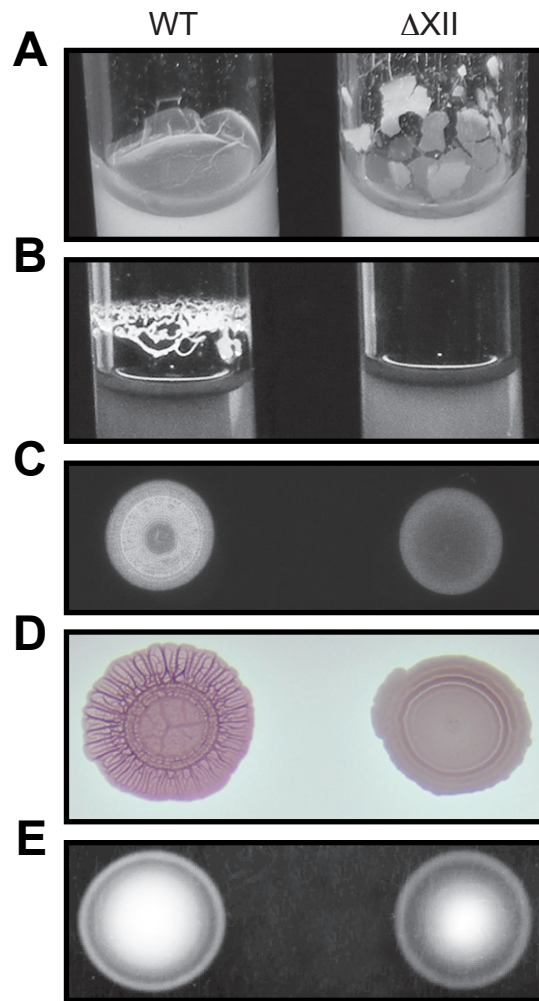d. NdeI site underlined
e. NotI site underlined

FIG S1 Characterization of the deletion present in ΔXII strain. Missing region in ΔXII strain described in (9) is delimted by red lines. This strain shows a deletion of 16,805 bp between nucleotides 2,935,768 and 2,952,573. *S.* Enteritidis P125109 genome was taken as reference.

FIG S2 Phenotypic analysis of new ΔXII strain. (A) Biofilm formation capacity in LB medium conditions and (B) adherence test medium (ATM) conditions. (C) Cellulose production on calcofluor agar plates. (D) Cellulose and fimbriae production on congo red agar plates. (E) Swimming motility. (F) Heading of genotype of sequential mutants that were constructed in the proccess of generating new ΔXII strain.

Supplemental references

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