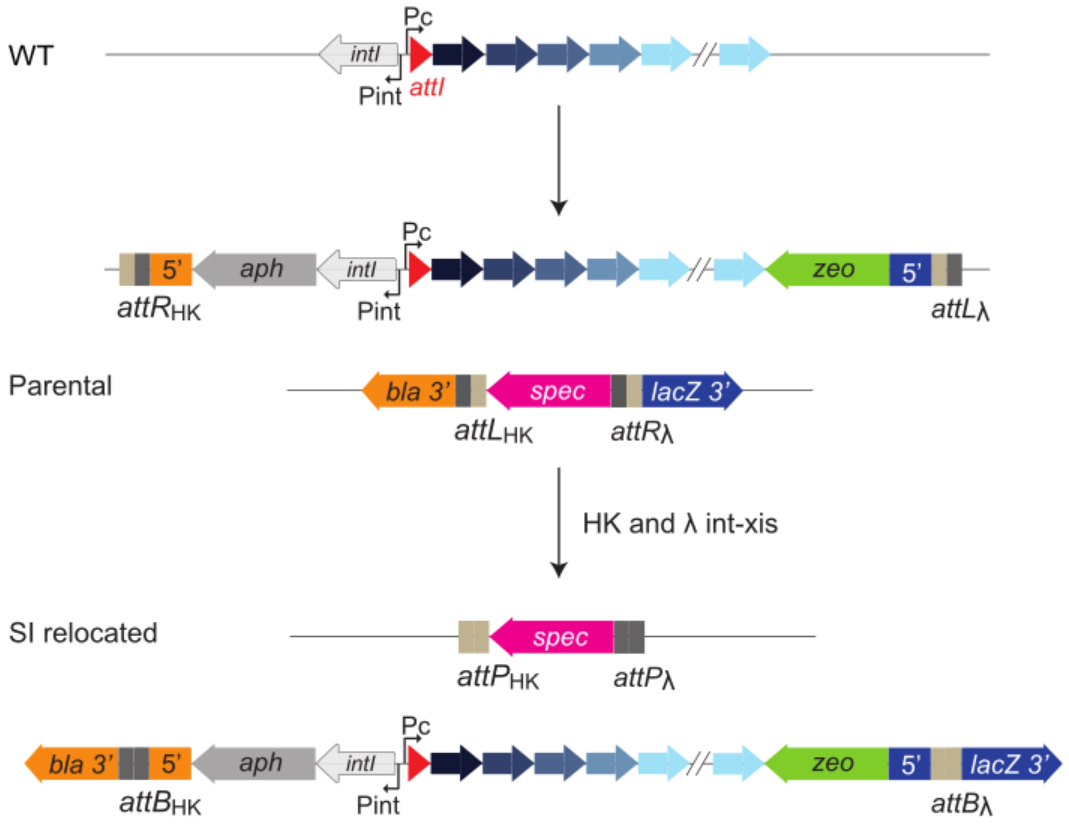


Figure S1. Schematic representation of the relocation of the *V. cholerae* SI from chromosome 2 to chromosome 1. The relocation is based on the recombination of two bacteriophage attachment sites, named *attL* and *attR*. These sites are associated with fragments of a genetic marker that becomes reconstituted when they recombine (*bla* and *lacZ*), allowing for the selection of those clones where the SI has been relocated.

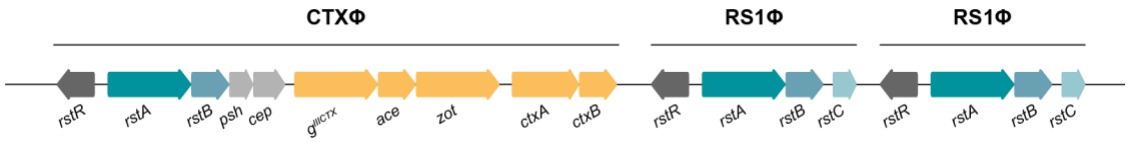


Figure S2. Schematic representation of the genetic region of the CTX Φ and RS1 Φ prophages in *V. cholerae* N16961. Our wild-type strain contains two copies of the RS1 Φ prophage that are composed of the *rstR*, *rstA*, *rstB,* and *rstC* genes. Only *rstR*, *rstA,* and *rstB* are present in the CTX Φ prophage genetic region.

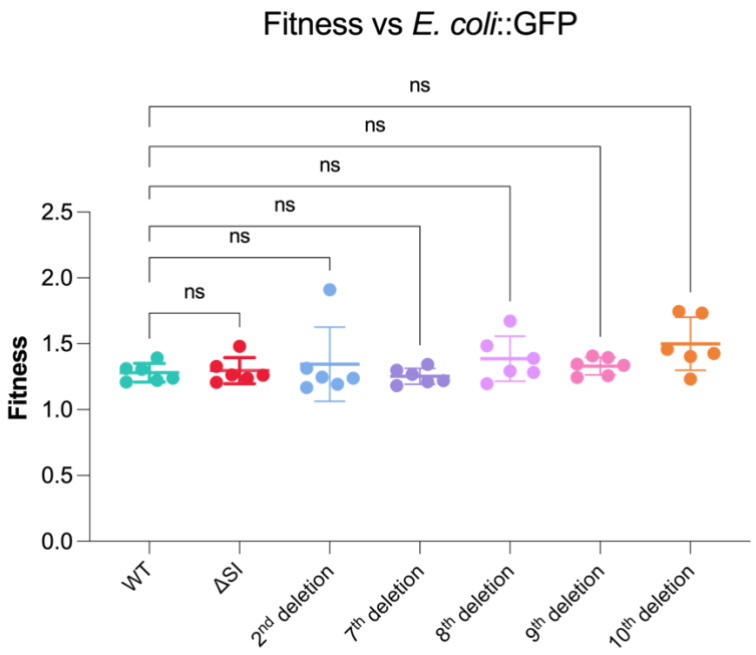
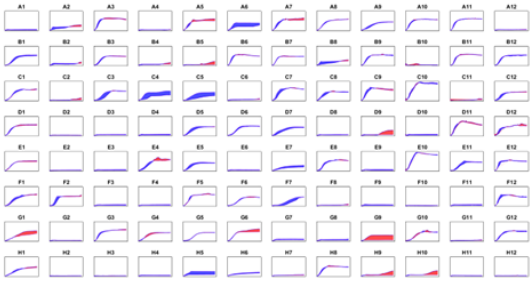


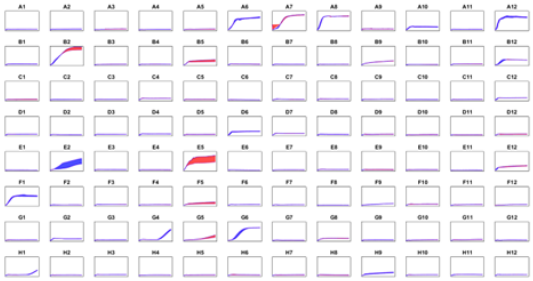
Figure S3. Competition assays of the intermediate strains generated in the deletion of the SI. Relative fitness of *V. cholerae* WT, ΔSI, and intermediate ΔSI strains compared with *E. coli* DH5α PcS::*gfp* was performed in LB by inoculating cells at a ratio of 1:1. Fitness values were determined from 6 independent experiments by flow cytometry. The p-values were calculated by comparing each measure with that of the WT strain using Dunnet's multiple comparison test. Ns: not significant.

Carbon sources

PM1



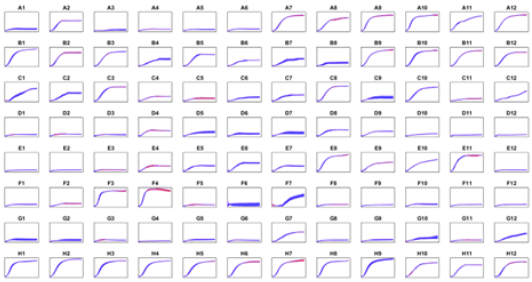
PM2



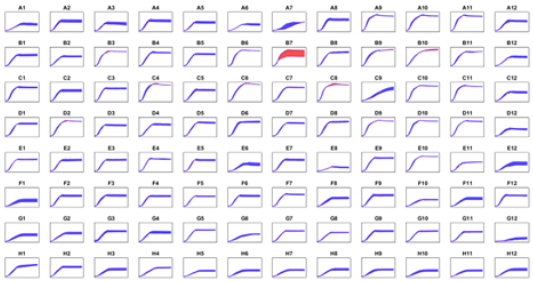
Nitrogen sources

Phosphorus and Sulfur sources

PM3



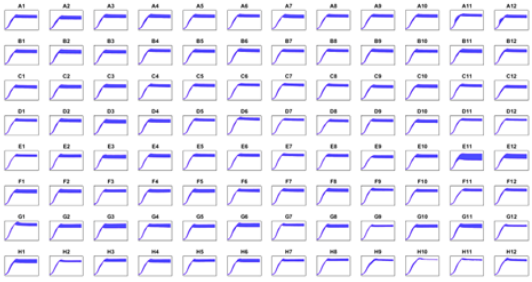
PM4



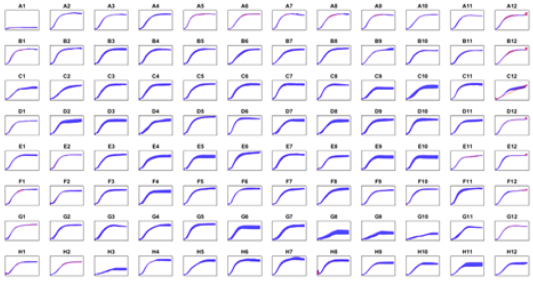
Nutrient supplements

Peptide Nitrogen sources

PM5

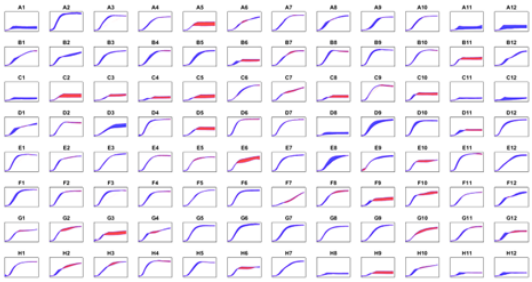


PM6

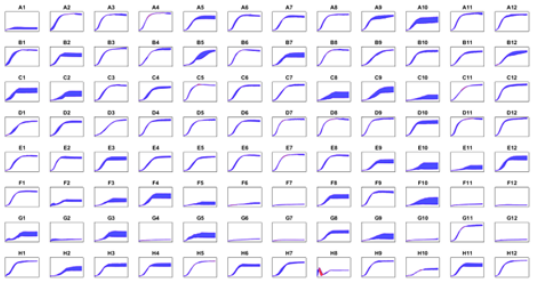


Peptide Nitrogen sources

PM7



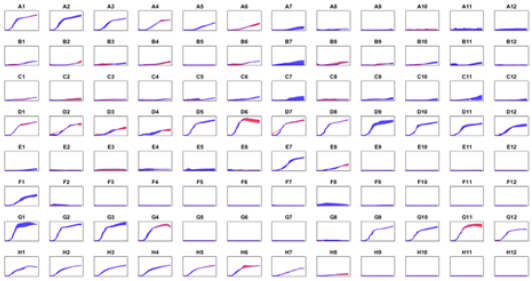
PM8



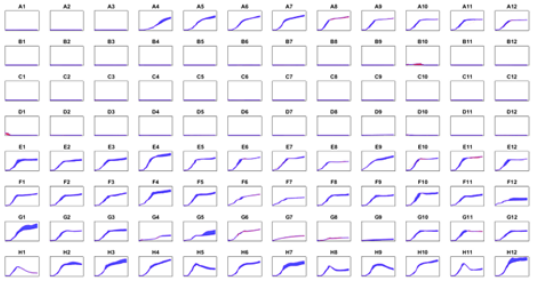
Osmolytes

pH

PM9

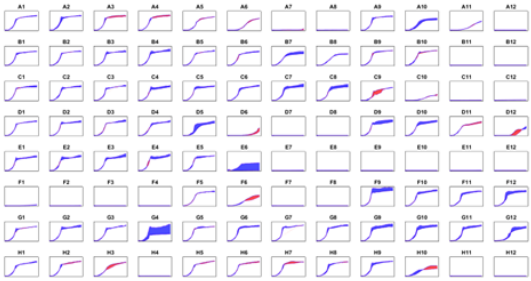


PM10

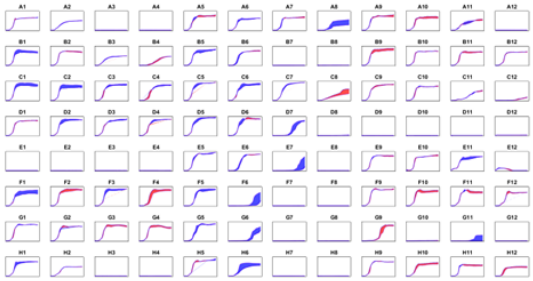


Antimicrobial compounds

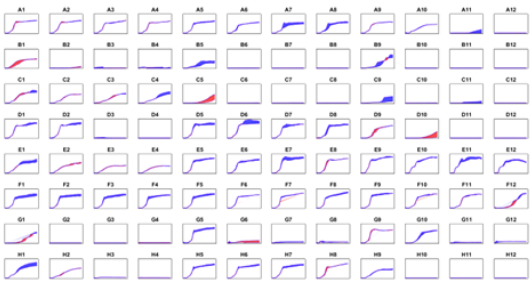
PM11



PM12



PM13



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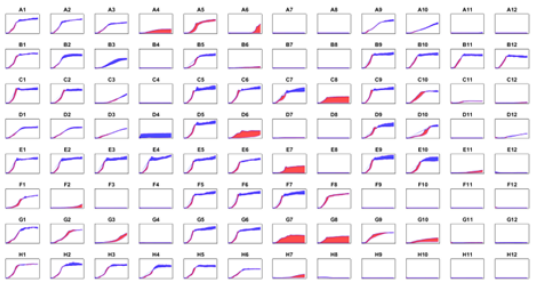
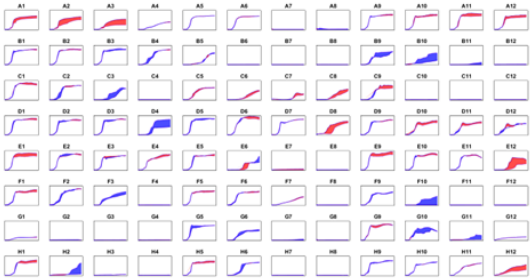


PM15



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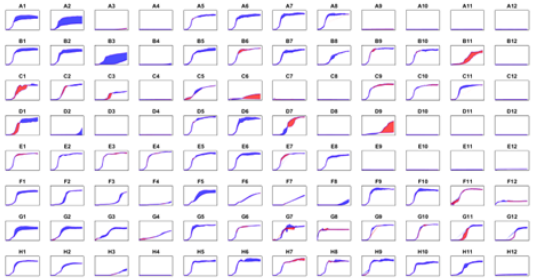

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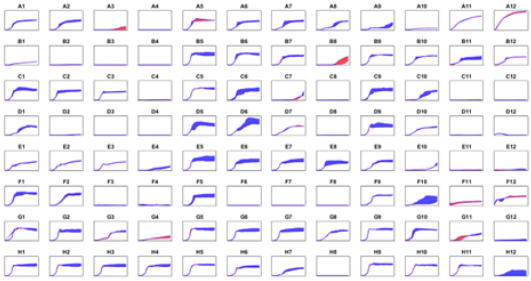

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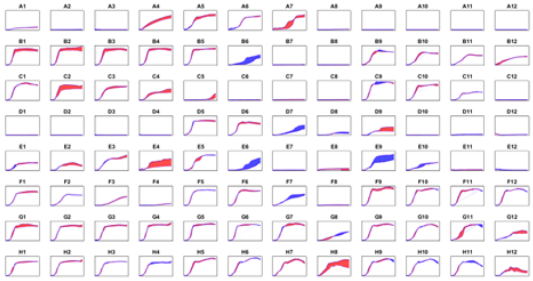

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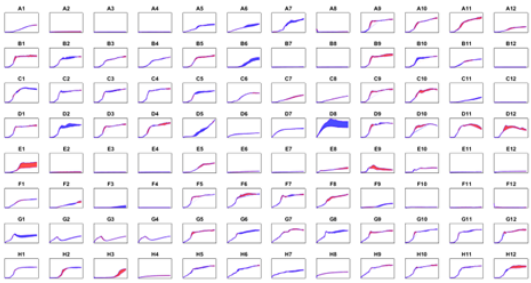

PM20

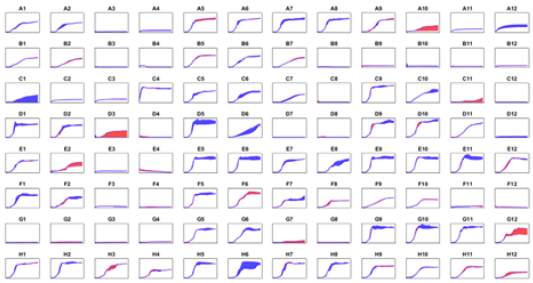

Figure S4. Growth curves representation of *V. cholerae* WT compared to the ΔSI mutant in all Biolog Phenotype microarrays plates (PM1-20). The mean growth curve from two independent biological replicates of the WT strain is graphically overlaid with the mean growth curve of the mutant strain calculated from two independent replicates. If the ΔSI mutant has an impaired growth in comparison with the WT, the overlapped area in the corresponding well in the graph will be blue, whereas if the ΔSI mutant has a growth improvement in comparison with the WT, the overlapped area in the corresponding well will be red.

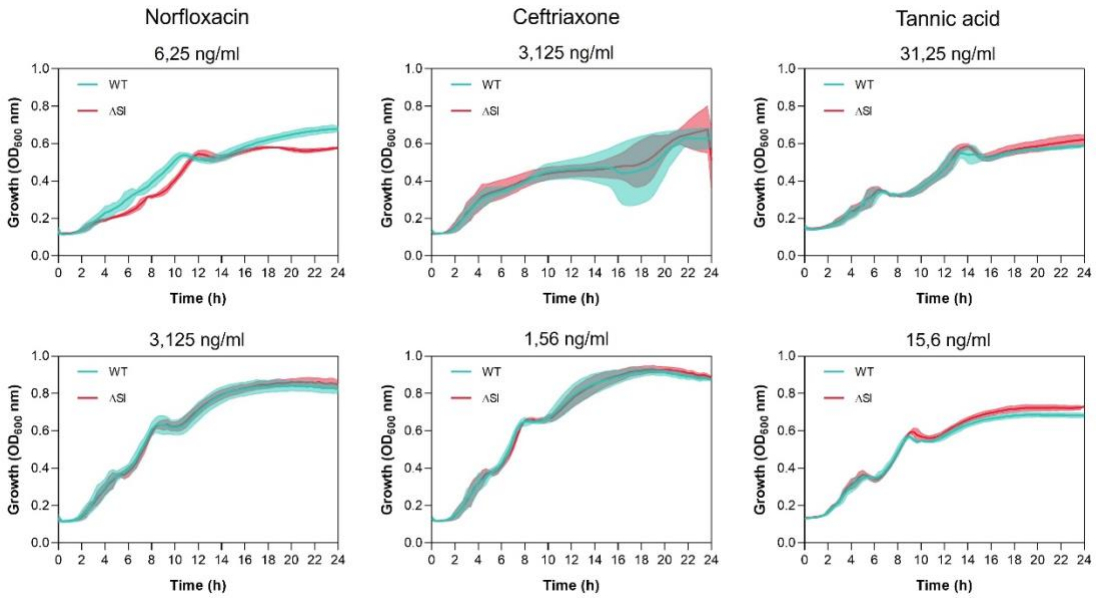

Figure S5. Growth curves of *V. cholerae* WT (blue) and ΔSI (red) strains in MH medium containing subinhibitory concentrations of norfloxacin, ceftriaxone, or tannic acid. Colored shading of the curves represents the standard deviation of at least three biological replicates.

Table S1. Strains generated for the relocation of the superintegron

Name	Genotype	Reference	Lab collection
8637	*V. cholerae* N16961 biovar El Tor, *hapR*+ (Tn::*hapR*) [StrepR] WT	Lab Collection, (1)	PGB
B805	8637 VC0018-*bla*3′-attL$_{HK}$-*cat*-attR$_{\lambda}$ *lacZ*-3′-VC0019	J. Bland, unpublished	PGB
B825	8637 VC0019-*bla*3′-attL$_{HK}$-*cat*-attR$_{\lambda}$ *lacZ*3′ -VC0018	J. Bland, unpublished	PGB
D282	8637 rplT-attR$_{HK}$-*bla*5′-*aph*	J. Garriss, unpublished	PGB
F854	B805 VC0018-*bla*3′-attL$_{HK}$-*aadA7*-attR$_{\lambda}$ *lacZ*3′-VC0019	This study, replacement by natural transformation of the *cat* fragment of B805 by the *spec* fragment from pF849	PGB
F855	B825 VC0019-*bla*3′-attL$_{HK}$-*aadA7*-attR$_{\lambda}$ *lacZ*3′ -VC0018	This study, replacement by natural transformation of the *cat* fragment of B825 by the *spec* fragment from pF849	PGB
F875	F854 rplT-attR$_{HK}$-*bla*5′-*aph-intIA*	This study, insertion by natural transformation of the *aph* fragment from pF851	PGB
F876	F855 rplT-attR$_{HK}$-*bla*5′-*aph-intIA*	This study, insertion by natural transformation of the *aph* fragment from pF851	PGB
J060, J061, J183	F875 VCA0508-*ble-lacZ*5′-attL$_{\lambda}$-VCA0510	(2), insertion by natural transformation of the *ble* fragment from pF912	PGB
J062, J063, J184	F876 VCA0508-*ble-lacZ*5′-attL$_{\lambda}$-VCA0510	(2), insertion by natural transformation of the *ble* fragment from pF912	PGB
J325, J327, J333	J060, J061 and J183 with pF930	(2), transformation of J060, J061 and J183 by pF930	PGB
J329, J331, J335	J062, J063 and J184 with pF930	(2), transformation of J062, J063 and J184 by pF930	PGB
J395, J399, J407	SCI relocated near ori1 (Chr1) betweenVC0018 and	(2), relocation by site-specific recombination of the SCI.	PGB

(SCI lag)	VC0019. *attCbs* carried by the lag strand template. From J329, J331, J335		
J387, J391, J403 **(SCI lead)**	SCI relocated near ori1 (Chr1) between VC0018 and VC0019. *attCbs* carried by the lead strand template. From J325, J327, J333	(2), relocation by site-specific recombination of the SCI.	PGB

PGB: Plasticité du Génome Bactérien Lab.

Table S2. Strains generated with SeqDelTA

Name	Genotype	Primers LHR	Primers RHR	Reference	Lab collection
A400	ΔVCA300-311. [Zeo^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 311 F – VCA 312 R	This study	MBA
A003	ΔVCA300-320. [Cm^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 318 F – VCA 320 R	This study	MBA
A004	ΔVCA300-325. [Carb^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 323 F – VCA 325 R	This study	MBA
A005	ΔVCA300-332. [Zeo^R]	VCA 299 F – LRHI VCA 300 R	LRHD + prom VCA 332 F – prom VCA 332 R VCA 333 F – VCA 334 R	This study	MBA
A006	ΔVCA300-350. [Carb^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 348 F – VCA 350 R	This study	MBA
A007	ΔVCA300-360. [Cm^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 359 F – VCA 360 R	This study	MBA
A009	ΔVCA300-385. [Zeo^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 311 F – VCA 386 R	This study	MBA
A023	ΔVCA300-391. [Carb^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 391 F – VCA 393 R	This study	MBA
A024	ΔVCA300-391 + VCA422-444. [Carb^R, Zeo^R]	VCA 421 F – LRHI VCA 422 R	LRHD VCA 443 F – VCA 447 R	This study	MBA
A029	ΔVCA300-470. [Cm^R]	VCA 299 F – LRHI VCA 300 R	LRHD + prom VCA 469 F – prom VCA 469 R VCA 469 F – VCA 470 R	This study	MBA
A035	ΔVCA300-470 + VCA474-483. [Cm^R, Zeo^R]	VCA 474 F – link AT/TA R / link AT/TA F – LRHI VCA 478 R	LRHD VCA 318 F – VCA 483 R	This study	MBA
A041	ΔVCA300-483. [Carb^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 318 F – VCA 483 R	This study	MBA
A047	ΔVCA300-483 + ΔVCA495-497. [Carb^R, Cm^R]	VCA 494 F – LRHI VCA 495 R	LRHD + prom VCA 497 F – prom VCA 497 R VCA 498 F – VCA 499-500 R	This study	MBA

A054	ΔVCA300-483 + ΔVCA487-505. [$Carb^R$, Zeo^R]	VCA 486 F – LRHI VCA 487 R	LRHD VCA 348 F – VCA 505 R	This study	MBA
A066	ΔVCA300-VCA505. [Zeo^R]	VCA 299 F – LRHI VCA 300 R	LRHD VCA 348 F – VCA 505 R	This study	MBA

MBA: Molecular Basis of Adaptation Lab.

Table S3. Parental strains and strains generated with pMP7

Name	Genotype	Reference	Lab Collection
A118	*E. coli* π3813, *ccdB*-resistant strain. (B410*gyrA*462zei::Tn10): *lacIQ*, *thi1*, *relA1*, *supE44*, *endA1*, *recA1*, *hsdR17*, *gyrA462*, *zei*298::Tn10, Δ*thyA*::(*erm-pir*116) [Erm^R] .Cloning of R6K vectors.	(3)	MBA
A116	*E. coli* β3914, *ccdB*-resistant strain. *gyrA462 zei298*::Tn10 [Erm^R, Km^R, Tc^R]. Conjugation of R6K vectors.	(3)	MBA
A097	*E. coli* β3914 pMP7_Δ *intIA attIA zeo*^{R}	This study	MBA
A631	*E. coli* π3813 pMP7-*rocS*$^+$	This study	MBA
A632	*E. coli* π3813 pMP7-*rpoS*$^+$	This study	MBA
A951	*E. coli* π3813 pMP7-*cry2*$^+$	This study	MBA
A633	*E. coli* β3914 pMP7-*rpoS*$^+$	This study	MBA
A634	*E. coli* β3914 pMP7-*rocS*$^+$	This study	MBA
A982	*E. coli* β3914 pMP7-*cry2*$^+$	This study	MBA
A001	*V. cholerae* N16961 biovar El Tor, *hapR*+ (Tn::*hapR*) [$Strep^R$]. WT (same as 8637, but stock from MBA lab).	Lab Collection, (1)	MBA
A096	*V. cholerae* N16961 biovar El Tor *hapR*+ (allele repaired *in situ*). WT	Lab Collection, (4)	MBA
A101	*V. cholerae* N16961 ΔSI	This study	MBA
A677	*V. cholerae* N16961 ΔSI *rocS*$^+$	This study	MBA
A684	*V. cholerae* N16961 ΔSI *rocS*$^+$ *rpoS*$^+$; pMP7_*rpoS*$^+$	This study	MBA
B522	ΔSI *V. cholerae* N16961 ΔSI *rocS*$^+$ *rpoS*$^+$ *cry2*$^+$; pMP7-_*cry2*$^+$	This study	MBA
B549	*V. cholerae* N16961 ΔVCA300-385 *rocS*$^+$	This study	MBA
B639	*V. cholerae* N16961 ΔVCA300-391 *rocS*$^+$	This study	MBA
B550	*V. cholerae* N16961 ΔVCA300-391 + VCA422-444 *rocS*$^+$	This study	MBA
B685	*V. cholerae* N16961 ΔVCA300-470 *rocS*$^+$	This study	MBA
A372	*E. coli* DH5α pSU38 P_CS::*gfp*	(5)	MBA
D318	*V. cholerae* N16961 ΔSI Δ*lacZ* $kana^R$	Lab Collection	MBA

MBA: Molecular Basis of Adaptation Lab.

Table S4. Plasmids used in this study

Name	Plasmid description	Relevant properties and construction	Lab collection
pA298	pFLP3 ::*aadA7*	[Sp^R] Val, unpublished	PGB
pJB6	pSU38Δ::attR$_{HK}$-attL$_{\lambda}$	*ori*P15A, [$Carb^R$] (6)	PGB
pA401	pSC101*rep*^{TS}:: *int$_{HK}$-xis$_{HK}$*	*oripSC101rep*^{TS} *oriT*$_{RP4}$; [Sp^R] (Bland, unpublished)	PGB
pF850	pTOPO::*VCA0508-ble-bla*3'-*att*LHK-*VCA0510*	*ori*ColE1; [Km^R] (2)	PGB
pF912	pTOPO-VCA0508-*ble-lacZ*5'-attL$_{\lambda}$-VCA0510	Assembly of three fragments by PCR. Fragments were amplified from pF850 with o4659 and o4708 primers, from pJB6 with o4707 and o4669 and, from 8637 with o4670 and o4662.	PGB
pF851	pTOPO-rplT-attR$_{HK}$-*bla*5'-*aph-intIA*	Assembly of three fragments by PCR. Fragments were amplified from 8637 with o4286 and o4672 primers, from D282 with o4671 and o4674 and, from 8637 with o4673 and o4302.	PGB
pF849	pTOPO- VC0018-*bla*3'attL$_{HK}$-*aadA7*-attR$_{\lambda}$ *lacZ*3'-VC0019	Assembly of three fragments by PCR. Fragments were amplified from B805 with o4665 and o4666, from pA298 with o4663 and o4664 and, from B805 with o4668 and o4667.	PGB
pF930	pA401 ::Cm^R	*oripSC101rep*^{TS} *oriT*$_{RP4}$; [Cm^R] insertion of the XhoI/KpnI digested *cat* fragment from pD060 in pA401.	PGB
pMP7	Suicide conjugative plasmid used for allelic exchange pSW23T-*araC* P$_{BAD}$-*ccdB*	*oriV*$_{R6K\gamma}$, *oriT*$_{RP4}$; [Cm^R]. (7)	MBA
pMP7_Δ *intIA attIA zeo^R*	pMP7-derivative used for performing the last deletion step of the superintegron (ΔVCA 291-300)	*oriV*$_{R6K\gamma}$, *oriT*$_{RP4}$; [Cm^R]. It contains two 500-bp fragments corresponding to each side of the superintegron. Fragments were amplified from *V. cholerae* N16961 gDNA with primers RHI link pMP7 F and LRHI+D R for the LHR, and RHD F and RHD link pMP7 R for the RHR.	MBA
pMP7-*rocS*$^+$	pMP7-derivative used for the correction of the *rocS* mutation	*oriV*$_{R6K\gamma}$, *oriT*$_{RP4}$; [Cm^R]. It contains a 1000-bp fragment of the *rocS* (VC0653) gene, amplified from *V. cholerae* N16961 gDNA with primers	MBA

		*rocS*_pMP7 F and *rocS*_pMP7 R.	
pMP7-*rpoS*$^{+}$	pMP7-derivative used for the correction of the *rpoS* mutation	$oriV_{R6K\gamma}$, $oriT_{RP4}$; [Cm^R]. It contains a 1000-bp fragment of the *rpoS* (VC0534) gene, amplified from *V. cholerae* N16961 gDNA with primers *rpoS*_pMP7 F and *rpoS*_pMP7 R.	MBA
pMP7-*cry2*$^{+}$	pMP7-derivative used for the correction of the *cry2* mutation	$oriV_{R6K\gamma}$, $oriT_{RP4}$; [Cm^R]. It contains a 1000-bp fragment of the *cry2* (VC01392) gene, amplified from *V. cholerae* N16961 gDNA with primers *cry2*_pMP7 F and *cry2*_pMP7 R.	MBA

PGB: Plasticité du Génome Bactérien Lab; **MBA**: Molecular Basis of Adaptation Lab.

Table S5. Primers used in this study

Name	Sequence (5′ → 3′)
Relocation of the superintegron	
4659	GAGTAAGTGTCGCTATCGAGGTGCTTCAGTCCTGCTCCTCGGCCACGAAG
4708	ACTTTTGGCGAAAATGAGACGTTGATATGCAATTGTCGGCACGTAAGAGG
4707	CCTCTTACGTGCCGACAATTGCATATCAACGTCTCATTTTCGCCAAAAGT
4669	CTTAGCCGACAATATCTGCCTATAAAATCAAATAATGATTTTATTTTGAC
4670	GTCAAAATAAAATCATTATTTGATTTTATAGGCAGATATTGTCGGCTAAG
4662	AGAAGTGCATGTTTCATCTCCCCCT
4286	GCTGTAGCACGTATGCTTCC
4672	ACTACTTAGATAGTATTAGTGACCTGGAATTCGTAATCATGGTCATAGCT
4671	AGCTATGACCATGATTACGAATTCCAGGTCACTAATACTATCTAAGTAGT
4674	ATTATCCCGTCTTTAGCATGGGTTCCGATCTCGCAGCGGTGGTAAGCGCC
4673	GGCGCTTACCACCGCTGCGAGATCGGAACCCATGCTAAAGACGGGATAAT
4302	AAGGTAAGGGGGGTAAAAATCGCAC
4665	GCCGGAAGGGCCGAGCGCAGAAGTG
4666	TCTAACAATTCGTTCAAGCCGACGCGACAAATGATTTTATTTTGACTAAT
4663	ATTAGTCAAAATAAAATCATTTGTCGCGTCGGCTTGAACGAATTGTTAGA
4664	GTGACCTGTAACAGAGCATTAGCGCCGAAACCTTGCGCTCGTTCGCCAGC
4668	GATCACACTCGGGTGATTACGATCG
4667	GCTGGCGAACGAGCGCAAGGTTTCGGCGCTAATGCTCTGTTACAGGTCAC
SeqDelTA	
3083	TCTAGGGCGGCGGATTTGTC
3327	TGCAATTGTCGGCACGTAAG
VCA 299 F	GGGCGTTAGAGCTTTATTGG
LRHI VCA 300 R	GACAAATCCGCCGCCCTAGAGAGCTTTATTTACTCGGACG
LRHD VCA 311 F	CTTACGTGCCGACAATTGCAGGTAAACGCTCAATCAAAGG
VCA 312 R	CTTTATTACGACAGCCATCGC
LRHD VCA 318 F	CTTACGTGCCGACAATTGCATGAAACGGTTCCTATCGTGC

VCA 320 R	CTGCCAAATCAGCATCAAGC
LRHD VCA 323 F	CTTACGTGCCGACAATTGCACAATGATGTCTGAAATCGGC
VCA 325 R	ACCTATGAGCTGACCAATGC
LRHD + prom VCA 332 F	CTTACGTGCCGACAATTGCAAAATATCACCTAACAAGCGC
prom VCA 332 R	CTGAGATGATCCTGACAATACCGTGCTTTTCGAG
VCA 333 F	AAAAGCACGGTATTGTCAGGATCATCTCAGTTCGG
VCA 334 R	TCATTGACCTCATCAAGACC
LRHD VCA 348 F	CTTACGTGCCGACAATTGCAATTGAGAGATGCTTCTTCCC
VCA 350 R	GAAACCTTCAGATAGCCGTC
LRHD VCA 359 F	CTTACGTGCCGACAATTGCATCACGTAAATCGGCTTTGGC
VCA 360 R	TGACCCTGCTCATTTCTTGC
LRHD VCA 311 F	CTTACGTGCCGACAATTGCAGGTAAACGCTCAATCAAAGG
VCA 386 R	ATACGATGGACGCCATAAGC
LRHD VCA 391 F	CTTACGTGCCGACAATTGCAGACTTAAGAATTCCACCGGG
VCA 393 R	TCAAGGTTAAGTTGGCCACG
VCA 421 F	GGTGATGCGACTCAAAAAGC
LRHI VCA 422 R	GACAAATCCGCCGCCCTAGATTGTAACGCTAGAGGTGACC
LRHD VCA 443 F	CTTACGTGCCGACAATTGCATTGAACTGCTGTTGGAGTGG
VCA 447 R	ATCGAGGGAAACGCATAACC
LRHD + prom VCA 469 F	CTTACGTGCCGACAATTGCAGCTCTTATCTGAACTAATTCTTGCC
prom VCA 469 R	GCCGTAAATGGTTAGCAAATCACTGAGATATTCATCTCGG
VCA 469 F	CCGAGATGAATATCTCAGTGATTTGCTAACCATTTACGGC
VCA 470 R	TGGTATAGAAGTCCTGTGCC
VCA 474 F	GGGCGTTATAACCTAATGGG
link AT/TA R	GGTAGTTGTTTTTTTGCGACAGCGGCCTTGGGAATATCAACGGC TCC
link AT/TA F	GGAGCCGTTGATATTCCCAAGGCCGCTGTCGCAAAAAAACAAC TACC
LRHI VCA 478 R	GACAAATCCGCCGCCCTAGAACTCCATTCCTTTTGCGCAG
LRHD VCA 318 F	CTTACGTGCCGACAATTGCATGAAACGGTTCCTATCGTGC
VCA 483 R	TTCCTGCCAATACTTGCTACC
VCA 494 F	TCGATGCTGCTTAACGGTGC
LRHI VCA 495 R	GACAAATCCGCCGCCCTAGAATCATGGCCATCAACTCTCC
LRHD + prom VCA 497 F	CTTACGTGCCGACAATTGCACGGGCGTTATATGCTTATAGG
prom VCA 497 R	GGATTTTCACCATTCACCGCATAGGGCATCAATCTCTGGC
VCA 498 F	GCCAGAGATTGATGCCCTATGCGGTGAATGGTGAAAATCC
VCA 499-500 R	TTGTGAGACTAAGCTGACCG
VCA 486 F	CCTATAATCGCTCAACTGACGG
LRHI VCA 487 R	GACAAATCCGCCGCCCTAGAACGGATTAGACTGACCTTTCC

VCA 505 R	CCTACACCGACAATGAAACC
RHI link pMP7 F	TCTGCGAGGCTGGCCGGCGTCCGTCAGCTGCGTCCAAACG
LRHI+D R	GCGACACTTACTCAAGCTGTCATGGGTTCTTTGCGAAATC
RHD F	ACAGCTTGAGTAAGTGTCGC
RHD link pMP7 R	TCAAGCTTATCGATACCGTCATTTGCTTTATGACTCGCGC
Correction of mutations	
***rocS*_pMP7 F**	CGATAAGCTTGATATCGAATTCCAGAAAAACCCTTGGTGTGC
***rocS*_pMP7 R**	CAGACAATTGACGGCTCTAGAATCAATCAGTTGAGGATTGC
***rpoS*_pMP7 F**	CGATAAGCTTGATATCGAATTCCGACGCGCAAGCACTTCTTT
***rpoS*_pMP7 R**	CAGACAATTGACGGCTCTAGAGTCAGCAATACCGTAACCA
***cry2*_pMP7 F**	GATAAGCTTGATATCGAATTCGTGAATCATAAGCGCGGTT
***cry2*_pMP7 R**	CAGACAATTGACGGCTCTAGAAACTCGGTAATAGGCAGAA
pMP7_bb_Gibson F	TCTAGAGCCGTCAATTGTCTG
pMP7_bb_Gibson R	GAATTCGATATCAAGCTTATCG
Natural transformation assay	
lacZ-FRT1	ATGCGCAACTTCTCCGATATTCTTCTTAGCC
lacZ-FRT4	GAGATACCACTTATCGCCCGCCACCAACTCG
RT-qPCR	
gyrA_rt_F	GAGCCAAAGTTACCTTGGCC
gyrA_rt_R	AATGTGCTGGGCAACGACTG
aspA_rt_F	TCTGCGGCGAATGTAATGGT
aspA_rt_R	TCCATCATGCCAGCGAAAGT
dcuA_rt_F	TGAAATCGGTGAAGCGGTGA
dcuA_rt_R	ACCGCATTTTCCACTTTGCC
rimO_rt_F	TGATTGTGTACCGGATGCGG
rimO_rt_R	TTAAAGTCTGGTCGTGCGCC
rstA_rt_F	ATCTGGGCGCCTCTTCCTAA
rstA_rt_R	CACCTCCAAGCGTTCCATCA
rstC_rt_F	GTTCAGGCGCTTATACAGACGA
rstC_rt_R	GTTGCGGATTTAGGCTTGGTG
vc02680_rt_F	CAAAACCCAATCCCACTGCG
vc02680_rt_R	CGTGGACTTGCAGAACTTTCG
vca02766_rt_F	TCCGACGTTAGACAAGTGGC
vca02766_rt_R	CCTTTCATCACCGGAGCACT

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