SUPPLEMENTARY MATERIAL

Supplementary Table S1. SadC (TM) mutants defective for interaction with MotC isolated in a bacterial two-hybrid screen.

Plasmid isolate	Point mutation*	Predicted location of mutation in full length SadC
1	**L82P**	TM3
2	**L94P**	TM3
3	**L134R**	TM5
4	L29P L148M	TM1 Between TM5 and TM6
5	L32Q V59E	TM1 TM2
6	W36R L152P	TM1 TM5
7	L66R F136S	TM2 TM5
8	W67R F139L	TM2 TM5
9	P85A S140P	TM3 TM5
10	L116P **F136Y**	TM4 TM5
11	G22D L57Q I91T	TM1 TM2 TM3
12	L37Q A133V	TM1 TM5
13	L66P	TM2
14	Q86H	TM3
15	L94Q F166Y	TM3 TM6
16	Y112C	TM4
17	L117P	TM4
18	L30P W171G	TM1 TM6

19	R20Q L63P	Before TM1 TM2
20	F121L S140P	TM4 TM5
21	L98P	TM3
22	L13P A133D	Before TM1 TM5
23	Y44F L134P	TM1 TM5
24	D79G	Between TM2 and TM3
25	Y44N T49S A90V I142F	TM1 Between TM1 and TM2 TM3 TM5
26	L104Q R130H I142T	TM4 TM5 TM5
27	Y77F L114Q L134Q S140P	Between TM2 and TM3 TM4 TM5 TM5

*Bolded alleles were retested for interaction with MotC and other phenotypes as described in the text.

Supplementary Table S2. Strains used in this study.

Strain Name	Genotype/Description	Source
***E. coli* strains**		
S17-1 λpir	*thi pro hsdR- hsdM+* Δ*recA* RP4-2::TcMu-Km::Tn7	(1)
BTH101	*F⁻, cya^{-99}, araD139, galE15, galK16, rpsL1 (Str^{r}), hsdR2, mcrA1, mcrB1*	Euromedex
S17-1 λpir	pmq30, SadC-3xFLAG KI construct, Gm^{R}	This study
S17-1 λpir	pmq30, SadC-3xFLAG L94P KI construct, Gm^{R}	This study
***P. aeruginosa* strains**		
SMC 232	PA14 wild type (WT)	(Rahme et al., 1995)
SMC 6365	WT (*gfp-motD*)	(2)
SMC 6366	Δ*bifA* (*gfp-motD*)	(3)
SMC 7562	Δ*bifA* Δ*motAB* (*gfp-motD*)	This study
SMC 3351	Δ*bifA*	(4)
SMC 5770	Δ*bifA* Δ*motAB*	(3)
SMC 5769	Δ*motAB*	(3)
SMC 5684	Δ*motCD*	(3)
SMC 7563	Δ*bifA* Δ*motCD*	This study
SMC 8238	SadC-3xFLAG	This study
SMC 8239	SadC-3xFLAG L94P	This study
SMC 8240	WT pMotAB	This study
SMC 7659	Δ*motCD* pmq72 empty vector	This study
SMC 8241	Δ*motCD* pMotAB	This study
SMC 4045	Δ*sadC* pmq72 empty vector	(5)
SMC 8242	Δ*sadC* pMotAB	This study

Supplementary Table S3. Plasmids used in this study.

Plasmid Name	Description	Source
pMQ30	Shuttle vector for yeast cloning and Gram-negative allelic replacement, Gm^r	(6)
pmq30	SadC-3xFLAG cloned into pmq30, Gm^R	This study
pmq30	SadC-3xFLAG L94P cloned into pmq30, Gm^R	This study
pMQ72	Shuttle vector for yeast cloning and arabinose-inducible gene expression, Gm^r	(6)
pKT25	BACTH vector allowing fusion to the C-terminus of the *cyaA* T25 fragment, Kan^r	Euromedex
pKNT25	BACTH vector allowing fusion to the N-terminus of the *cyaA* T25 fragment, Kan^r	Euromedex
pUT18	BACTH vector allowing fusion to the N-terminus of the *cyaA* T18 fragment, Amp^r	Euromedex
pUT18C	BACTH vector allowing fusion to the C-terminus of the *cyaA* T18 fragment, Amp^r	Euromedex
pKT25-zip	Leucine zipper of GCN4 fused to T25 in pKT25, Kan^r	Euromedex
pUT18C-zip	Leucine zipper of GCN4 fused to T18 in pUT18C, Amp^r	Euromedex
pUT18C-*sadC*	Full length *sadC* cloned into pUT18C, Amp^r	This study
pUT18C-*roeA*	Full length *roeA* cloned into pUT18C, Amp^r	This study
pKT25-*motA*	Full length *motA* with a C-terminal 6xHis tag cloned into pKT25, Kan^r	(7)
pKT25-*motC*	Full length *motC* cloned into pKT25, Kan	(7)
pUT18C-	Transmembrane domain of *sadC* (amino acids 1-187)	This study

sadC(TM)	cloned into pUT18C, Amp^r	
pUT18C-*sadC*(cyto)	Cytoplasmic domain of *sadC* (amino acids 188-375) cloned into pUT18C, Amp^r	This study
pUT18C-*roeA*(TM)	Transmembrane domain of *roeA* (amino acids 1-197) cloned into pUT18C, Amp^r	This study
pUT18C-*roeA*(cyto)	Cytoplasmic domain of *roeA* (amino acids 1-187) cloned into pUT18C, Amp^r	This study
pUT18C-*sadC*(L82P)	*sadC*(L82P) cloned into pUT18C, Amp^r	This study
pUT18C-sadC(L94P)	*sadC*(L94P) cloned into pUT18C, Amp^r	This study
pUT18C-sadC(L134R)	*sadC*(L134R) cloned into pUT18C, Amp^r	This study
pKT25-sadC(L82P)	*sadC*(L82P) cloned into pKT25, Kan^r	This study
pKT25-sadC(L94P)	*sadC*(L94P) cloned into pKT25, Kan^r	This study
pKT25-sadC(L134R)	*sadC*(L134R) cloned into pKT25, Kan^r	This study

Supplementary Table S4. Oligonucleotide primers used in this study.

Primer Name	Primer Sequence*
B2H-sadC-F	NNNNNNTCTAGAGATGCGCACAGACAAGCCTC
B2H-sadC-R	NNNNNNGGATCCTCGGCACTGGTGACCTCCCA
SadC 1-187 BTH R	GGCGGGATCCTCGCGCATGCGTTGCCGCATC
SadC 188-375 BTH F	GGCGTCTAGAGCAGCGCCGCTATGCCTTG
SadC 188-375 BTH R	GGCGGGATCCTCGGCACTGGTGACCTCCCAG
RoeA 1-197 BTH R	GCGGGGATCCTCCCCGTGCCAGAGGATCAG
RoeA 198-398 BTH F	GGCGTCTAGAGCACGTGCGCAACCTGCGC
RoeA 198-398 BTH R	GGCGGGATCCTCCCGCAGGCTTTCCGCGAG
SadC L82P For	GTTACGCCGATCCCAGCCCGACCGAGCCGCAGGTGC
SadC L82P Rev	GCACCTGCGGCTCGGTCGGGCTGGGATCGGCGTAAC
SadC L94P For	GGTGGCGATCGCCTGGCCGACCTATTTCCTCTATCACGTC
SadC L94P Rev	GACGTGATAGAGGAAATAGGTCGGCCAGGCGATCGCCAC C
SadC L134R For	CGCCCGCTGTGCGGCGCGGGCGTTCATCGC
SadC L134R Rev	GCGATGAACGCCCGCGCCGCACAGCGGGCG
SadC F136Y For	CGCTGTGCGGCGCTGGCGTACATCGCTTTTTCCG
SadC F136Y Rev	CCGGAAAAAGCGATGTACGCCAGCGCCGCACAGC
B2H-MotA For	GGCGTCTAGAGATGTCAAAAATCATCGGCATCATCG
B2H-MotA Rev (used a His tagged template for this amplification)	GGCG GGATCC TC **GTGGTGATGGTGGTGGTG**
B2H-MotC For	GGCGTCTAGA G ATGGATGTGCTCAGCCTGGTC

B2H-MotC Rev	GGCGGGATCC TC GTCCATGAAGCCTTGCAGC
SadC native RBS pMQ72 F	caactctctactgtttctccatacccgttttttgggTCTTCAGGCGGGTAATTCG AATG
SadC-His pMQ72 R	taatctgtatcaggctgaaaatcttctctcatccgcctca**GTGGTGATGGTGGTG GTG**GGCACTGGTGACCTCCCAGG
Linker + 3x FLAG G-Block sequence	GGCGGCAGCGGCGGCGGCAGCGGCGGCGACTACAAAGACCATGACG GTGATTATAAAGATCATGATATCGACTACAAAGATGACGACGATAAA TAG
SadC-3x FLAG KI F_1 upstream	tgtaaaacgacggccagtgccaagcttgcatgcctgCCGATGCCCAGCTGGTTGACC
SadC-3x FLAG KI R_2	CGCTGTTTCAGAGGAGGCTTGTCTGTGCGCATGGGCTCCGTCC CGTAATGGCACCTGG
SadC-3x FLAG KI F_3	CGACTACAAAGATGACGACGATAAATAGGTGCCTGACATACG GGTCGGCGAGCGACGTC
SadC-3x FLAG KI F_4 downstream	ccatgattacgaattcgagctcggtacccggggatccTCAGGCGTGGGGCAGGAACA G

* In primer sequences, uppercase boldface letters indicate a 6xHis tag, lowercase letters indicate sequence complementary to pMQ72, and underlined letters indicate point mutations.

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