Surface sensing and lateral subcellular localization of WspA, the receptor in a chemosensory-like system leading to c-di-GMP production.

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Table S1: Bacterial strains used in this study.

***Pseudomonas aeruginosa* strains**	**Genotype**	**Reference/source**
PAO1	Wild-type	(Stover *et al.*, 2000)
PAO1 *mCherry-mreB*	PAO1*attB::*pSW*mCherry-mreB*	(Cowles & Gitai, 2010)
PAO1100	Δ*wspF*	(Hickman et al., 2005)
PAO1101	Δ*wspA*	(Hickman et al., 2005)
PAO1251	*wspR–yfp*	(Guvener & Harwood, 2007)
PAO1252	PAO1101, *wspR–yfp*	(Guvener & Harwood, 2007)
PAO1254	Δ*wspF wspR–yfp*	(Guvener & Harwood, 2007)
PAO1260	Δ*wspA,* Δ*wspF wspR–yfp*	(Guvener & Harwood, 2007)
PAO1275	*wspA–yfp*	(Guvener & Harwood, 2007)
PAO1276	Δ*wspE wspA–yfp*	(Guvener & Harwood, 2007)
CSH25	PAO1251 Δ*wspC*	This study
CSH27	PAO1254 Δ*wspC*	This study
CSH75	PAO1251 *pctA-wspA*	This study
CSH76	PAO1254 *pctA-wspA*	This study
CSH169	PAO1251 *wspC*ΔTPR	This study
CSH171	PAO1254 *wspC*ΔTPR	This study
CSH199	PAO1251 *wspE*ΔREC	This study
CSH252	PAO1254 *wspE*ΔREC	This study
CSH285	PAO1 *wspA*-CFP	This study
CSH292	CSH75 *pctA-wspA-cfp*	This study
CSH295	CSH76 *pctA-wspA-cfp*	This study
CSH366	PAO1251 *pctC-wspA*	This study
CSH368	PAO1254 *pctC-wspA*	This study
CSH373	PAO1251 PA2652-*wspA*	This study
CSH375	PAO1254 PA2652-*wspA*	This study
CSH377	PAO1251 *pctB-wspA*	This study
CSH379	PAO1254 *pctB-wspA*	This study
CSH382	CSH366 *pctC-wspA-cfp*	This study
CSH383	CSH368 *pctC-wspA-cfp*	This study
CSH385	CSH373 PA2652-*wspA-cfp*	This study
CSH386	CSH375 PA2652-*wspA-cfp*	This study
CSH387	CSH377 *pctB-wspA-cfp*	This study
CSH388	CSH379 *pctB-wspA-cfp*	This study
CSH448	PAO1 *pctB-yfp*	This study
CSH477	PAO1 *pctA-yfp*	This study
CSH504	PAO1 *wspA-pctA-yfp*	This study
CSH627	PAO1275 Δ*wspD*	This study
CSH628	PAO1254 Δ*wspD*	This study
CSH676	PAO1101*attB::*MINICTX2	This study
CSH687	PAO1101*attB::*MINICTX2-*wspA-yfp*	This study
CSH691	PAO1101*attB::*MINICTX2-*wspA*-5 -*yfp*	This study
CSH704	PAO1251 Δ*wspB*	This study
CSH705	PAO1254 Δ*wspB*	This study
CSH709	PAO1275 Δ*wspB*	This study
CSH712	PAO1251 Δ*wspD*	This study
CSH954	CSH687 Δ*wspE*	This study
CSH956	CSH691 Δ*wspE*	This study

***Escherichia coli* strains**	**Characteristics/Genotype**	**Reference/source**
DH5α	Used for cloning purposes	Gibco BRL
S17-1	Donor strain for conjugal transfer of plasmids to *P. aeruginosa*.	(Simon *et al.*, 1983)
NEB5α *F' lacI*^{q}	Used for cloning purposes	NEB
HCB437	Chemotaxis deficient strain: Δ(*tsr*)7021 Δ(*trg*)100 zbd::TnS Δ(*cheA-cheZ*)2209 *metF*159(Am)	(Wolfe *et al.*, 1987)
RP437	Chemotaxis proficient strain background	(Parkinson, 1978)
CSH658	HCB437 (MINICTX2)	This study
CSH659	HCB437 (MINICTX2-*wspA-yfp*)	This study
CSH661	HCB437 (MINICTX2-*wspA*-5 -*yfp*)	This study
CSH737	RP437 (MINICTX2)	This study
CSH741	RP437 (MINICTX2-*wspA-yfp*)	This study
CSH745	RP437 (MINICTX2-*wspA*-5 -*yfp*)	This study

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Table S2: Plasmids used in this study

Plasmid name	Use/Characteristics	Reference/source
pEX18Gm	*P. aeruginosa* suicide vector for construction of in-frame insertion/deletion/replacement mutants.	(Hoang *et al.*, 1998)
pEX19Gm	*P. aeruginosa* suicide vector for construction of in-frame insertion/deletion/replacement mutants.	(Hoang et al., 1998)
pEX18Gm *wspA-pctA*	For replacement of *wspA* with *wspA-pctA* fusion in native *wspA* location on chromosome. The 5' end of *wspA* (encoding $WspA_{1-258}$) was fused to 3' end of *pctA* (encoding $PctA_{346-629}$). 5' *wspA* amplified with primers CHP248/250, 3' *pctA* amplified with CHP251/252, *wspB* amplified with CHP253/249. The three PCR products were fused together and inserted *Hin*dIII/*BamHI* into pEX18Gm using In Fusion cloning kit.	This study
pEX18Gm *wspA-pctA-yfp*	For construction of *wspA-pctA-yfp* fusion in *wspA* native location in *wsp* locus. *pctA* fragment PCR amplified with CHP246/244, 5-glycine linker-*yfp* fragment amplified from pEX19GmEYFP with CHP173/174, *wspB* fragment amplified withCHP270/249. Fragments fused together and inserted into *Hin*dIII/*BamHI* sites of pEX18Gm using In Fusion cloning.	This study
pEX18Gm*wspE*ΔREC in Δ*wspF* background	Suicide vector to generate in-frame deletion of *wspE* REC domain. Upstream *wspE* REC PCR amplified with CHP87/90, downstream *wspE* REC amplified with CHP89/*wspF* reverse. SOE PCR product amplified with CHP87/*wspF*reverse and cloned *Hin*dIII/ *Bam*HI into the same sites of pEX18Gm.	This study
pEX19Gm *pctA-yfp*	For construction of chromosomal fusion of *yfp* to 3' end of *pctA* in native *pctA* location. *pctA* fragment amplified with CHP246/244, 5-glycine linker-*yfp* fragment amplified from pEX19GmEYFP with CHP173/174, DNA downstream of *pctA* amplified with CHP245/247. Fragments fused together and inserted into *Hin*dIII/*BamHI* sites of pEX19Gm using In Fusion cloning.	This study
pEX19Gm *pctA-wspA*	For replacement of *wspA* with *pctA-wspA* fusion in native *wspA* location on chromosome. The 5' end of *pctA* (encoding $PctA_{1-345}$) was fused to 3' end of *wspA* (encoding $WspA_{259-542}$). *wspA* upstream region amplified with primers CHP13/59, 5' *pctA* amplified with CHP58/61, 3' *wspA* amplified with CHP60/14. The three PCR were products fused together and inserted *Hin*dIII/*BamHI* into pEX19Gm using In Fusion cloning kit.	This study
pEX19Gm *pctB-wspA*	For replacement of *wspA* with *pctB-wspA* fusion in native *wspA* location on chromosome. The 5' end of *pctB* (encoding $PctB_{2-345}$) was fused to 3' end of *wspA* (encoding $WspA_{259-542}$). *wspA* upstream region amplified with primers CHP13/211, 5'*pctB* amplified with CHP213/214, 3' *wspA* amplified with CHP212/14. The three PCR products were fused together and inserted	This study

	*Hin*dIII/*BamHI* into pEX19Gm using In Fusion cloning kit.	
pEX19Gm *pctC-wspA*	For replacement of *wspA* with *pctC-wspA* fusion in native *wspA* location on chromosome. The 5' end of *pctC* (encoding $PctC_{2-348}$) was fused to 3' end of *wspA* (encoding $WspA_{259-542}$). *wspA* upstream region amplified with primers CHP13/211, 5'*pctC* fragment amplified with CHP215/216, 3'*wspA* amplified with CHP212/14. The three PCR products were fused together and inserted *Hin*dIII/*BamHI* into pEX19Gm using In Fusion cloning kit.	This study
pEX19Gm PA2652-*wspA*	For replacement of *wspA* with PA2652-*wspA* fusion in native *wspA* location on chromosome. The 5' end of PA2652 (encoding $PA2652_{2-277}$) was fused to 3' end of *wspA* (encoding $WspA_{259-542}$). *wspA* upstream region amplified with primers CHP13/211, 5' PA2652 amplified with CHP217/218, 3'*wspA* amplified with CHP212/14. The three PCR products were fused together and inserted *Hin*dIII/*BamHI* into pEX19Gm using In Fusion cloning kit.	This study
pEX19Gm *pctB-yfp*	For construction of *pctB-yfp* fusion in native *pctB* location on chromosome. *pctB* was PCR amplified with primers pctB_F and pctB_R, then cloned *Eco*RI/*Kpn*I into the same sites of pEX19Gm EYFP, resulting in pEX19Gm *pctBUP-yfp*. Then the region downstream of *pctB* was PCR amplified with primers pctBdown_F/pctBdown_R and cloned *Xba*I/*Hin*dIII into the same sites of pEX19Gm *pctBUP-yfp*, resulting in pEX19Gm *pctB-yfp*.	This study
pEX19Gm Δ*wspB* (for *wspB* deletion in a background with WT *wspA*)	Suicide vector to construct in-frame deletion of *wspB* in WT *wspA* background. Upstream *wspB* PCR amplified with CHP339/342, downstream *wspB* PCR amplified with CHP341/340. SOE PCR product amplified with CHP339/340 and cloned *Kpn*I/*Hin*dIII into the same sites of pEX19Gm.	This study
pEX19Gm Δ*wspB* (for *wspB* deletion in a background with *wspA-yfp*)	Suicide vector to construct in-frame deletion of *wspB* in a *wspA-yfp* background. Upstream *wspB* PCR amplified with CHP167/ WspB-YFP-upR from PAO1275, downstream *wspB* PCR amplified with WspBdownF/CHP340. SOE PCR product amplified with CHP167/340 and cloned Blunt/*Hin*dIII into the *Xmn*I/*Hin*dIII sites of pEX19Gm.	This study
pEX19GmΔ*wspC*	Suicide vector to generate in-frame deletion of *wspC*. Upstream *wspC* PCR amplified with CHP1/48, downstream *wspC* amplified with CHP47/4. SOE PCR product amplified with CHP1/4 and cloned *Hind*III/*BamH*I into the same sites of pEX19Gm.	This study
pEX19Gm*wspC*ΔTPR	Suicide vector to generate in-frame deletion of *wspC* TPR domain. Upstream *wspC*TPR PCR amplified with CHP102/104, downstream *wspC*TPR amplified with CHP103/4. SOE PCR product amplified with CHP102/4 and cloned *Hind*III/*BamH*I into the same sites of pEX19Gm.	This study
pEX19GmΔ*wspD*	Suicide vector to generate in-frame deletion of *wspD*. Upstream *wspD* PCR amplified with CHP303/323, downstream *wspD* amplified	This study

	with CHP322/304. SOE PCR product amplified with CHP303/304 and cloned *Bam*HI/*Hin*dIII into the same sites of pEX19Gm.	
pEX19GmΔ*wspE*	Suicide vector for in-frame deletion of *wspE*	(Hickman et al., 2005)
pEX19Gm*wspE*ΔREC	Suicide vector to generate in-frame deletion of *wspE* REC domain. Upstream *wspE* REC PCR amplified with CHP87/90, downstream *wspE* REC amplified with CHP89/88. SOE PCR product amplified with CHP87/88 and cloned *Hin*dIII/ *Bam*HI into the same sites of pEX19Gm.	This study
pEX19GmEYFP	Cloning vector for construction of *eyfp* fusion suicide vectors.	(Guvener et al., 2006)
pEYFP	Cloning/expression vector encoding EYFP	Clontech
pEX19Gm *wspA-yfp*	For construction of *wspA-yfp* fusions in native *wspA* location on the chromosome.	(Guvener & Harwood, 2007)
pEX19Gm *wspA-cfp*	For construction of *wspA-cfp* fusions in native *wspA* location on the chromosome.	(Guvener & Harwood, 2007)
pUC57-3' *wspA*-4 mutations	Synthetic *wspA* 3' fragment, encoding 4 amino acid mutations, cloned *Eco*RI/*Hin*dIII into pUC57. See fig. S3 for description of mutations.	Genewiz Inc.
MINICTX2	*E. coli-P. aeruginosa* shuttle vector that integrates into the *attB* site of *P. aeruginosa* by site-specific recombination.	(Hoang et al., 2000)
MINICTX2-*wspA-yfp*	PCR amplified *wspA* including native promoter using primers CHP336/292, PCR amplified 5-glycine linker-*yfp* from pEYFP using primers CHP173/335, fragments fused together and inserted into MINICTX2 *Bam*HI/*Kpn*I using In Fusion cloning.	This study
MINICTX2-*wspA*	PCR amplified *wspA* including native promoter using primers CHP336/334 and cloned *Bam*HI/*Kpn*I into the same sites of MINICTX2.	This study
MINICTX2-*wspA*-5-*yfp*	PCR amplified *wspA* 5' and native promoter using primers CHP336/338, PCR amplified 3' *wspA*-4 mutations from pUC57-3'*wspA*-4 mutations using CHP337/334. *wspA*-5 mutations SOE PCR product then generated with CHP336/292, which excluded the stop codon. PCR amplified 5-glycine linker-*yfp* from pEYFP using primers CHP173/335. SOE PCR and 5-glycine linker-*yfp* fragment fused together and inserted into MINICTX2 *Bam*HI/*Kpn*I using In Fusion cloning. See Fig. S3 for detailed description of the location of the 5 mutations.	This study
MINICTX2-*wspA*-5	PCR amplified wspA-5 including native promoter from MINICTX2-*wspA*-5-*yfp* using CHP336/334. Product cloned *Kpn*I/*Bam*HI into the same sites of MINICTX2. See Fig. S3 for detailed description of the location of the 5 mutations.	This study

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11 **Table S3: Primers used in this study.**

Primer number	Sequence	Use	Source/ Reference
CHP1	5'-TTTAAAGCTTAAGTCTGAGGCTGTGACCGTGT-3'	Construction of pEX19GmΔ*wspC*	This study
CHP4	5'-AATTGGATCCCGGAAATGATCAGCATGCGCGGAACC-3'	Construction of pEX19GmΔ*wspC* and pEX19Gm*wspC*ΔTPR	This study
CHP13	5'-TATGACCATGATTACGCCAAGCTTCTGGCGGCGCTGATGGTCGAACTGTT-3'	Construction of pEX19Gm *pctA-wspA*, pEX19Gm *pctB-wspA,* pEX19Gm *pctC-wspA,* pEX19Gm PA2652-*wspA*	This study
CHP14	5'-TTCGAGCTCGGTACCCGGGGATCCTCAGACTTTGAAGCGGGATACG-3'	Construction of pEX19Gm *pctA-wspA*, pEX19Gm *pctB-wspA,* pEX19Gm *pctC-wspA,* pEX19Gm PA2652-*wspA*, pPSV35-*wspA*	This study
CHP47	5'-GGGACGGCATGAACGATCGCTTCCAGCAACGTGCGGCCCGTGGAG-3'	Construction of pEX19GmΔ*wspC*	This study
CHP48	5'-CTCCACGGGCCGCACGTTGCTGGAAGCGATCGTTCATGCCGTCCC-3'	Construction of pEX19GmΔ*wspC*	This study
CHP58	5'-CGGACTCGAGTCTTGGGGAAAAATATGATCAAAAGTCTGAAGTTCAGC-3'	Construction of pEX19Gm *pctA-wspA*	This study
CHP59	5'-GCTGAACTTCAGACTTTTGATCATATTTTTCCCCAAGACTCGAGTCCG-3'	Construction of pEX19Gm *pctA-wspA*	This study
CHP60	5'-GATGCCTTCAACCAGTTCGTCGAGGAGCTCAAGGGCCTGGTGTCGCAG-3'	Construction of pEX19Gm *pctA-wspA*	This study
CHP61	5'-CTGCGACACCAGGCCCTTGAGCTCCTCGACGAACTGGTTGAAGGCATC-3'	Construction of pEX19Gm *pctA-wspA*	This study
CHP87	5'-AATTTAAGCTTTGTTTCACGTCGAGGTGCCGCTGAC-3'	pEX19Gm*wspE*ΔREC	This study
CHP88	5'-TTTAAGGATCCTTGCTTGAGCAACACCGCGAGCGAC	pEX19Gm*wspE*ΔREC	This study
CHP89	5'-GCGGGGCGCAACGCAAGCGCGTCGTCGTCCTGATCGGAGA-3'	pEX19Gm*wspE*ΔREC	This study
CHP90	5'-TCTCCGATCAGGACGACGACGCGCTTGCGTTGCGCCCCGC-3'	pEX19Gm*wspE*ΔREC	This study
CHP102	5'-AATTAAAGCTTCCAGTTGGCCGAGCAGGTTC-3'	pEX19Gm*wspC*ΔTPR	This study
CHP103	5'-GCGGCCAGCCCCTCGCCAGTCAGCAACGTGCGGCCCGTGG-3'	pEX19Gm*wspC*ΔTPR	This study
CHP104	5'-CCACGGGCCGCACGTTGCTGACTGGCGAGGGGCTGGCCGC-3'	pEX19Gm*wspC*ΔTPR	This study
CHP167	5'-GTGAGCAAGGGCGAGGAGCTGTTC-3'	Construction of pEX19Gm Δ*wspB* (for *wspB* deletion in a background with *wspA-yfp*)	This study
CHP173	5'-GGCGGTGGCGGTGGCGTGAGCAAGGGCGAGGAG-3'	Construction of MINICTX2-*wspA-yfp* and MINICTX2-*wspA*-5-*yfp*	This study
CHP174	5'-CTTGTACAGCTCGTCCATGCCGAGA-3'	Construction of pEX19Gm *pctA-yfp* and pEX18Gm *wspA-pctA-yfp*	This study
CHP211	5'-CACATTTTTCCCCAAGACTCGAGTCCGGATTCGTTCCTG-3'	Construction of pEX19Gm *pctB-wspA,* pEX19Gm *pctC-wspA* and pEX19Gm PA2652-*wspA*	This study

CHP212	5′-GAGCTCAAGGGCCTGGTGTCGCAGG-3′	Construction of pEX19Gm *pctB-wspA,* pEX19Gm *pctC-wspA* and pEX19Gm PA2652-*wspA*	This study
CHP213	5′-TTGGGGAAAAATGTGATCAAAAGTCTCAAGTTCAGCCA-3′	Construction of pEX19Gm *pctB-wspA*	This study
CHP214	5′-CAGGCCCTTGAGCTCCTCGACGAAGCGGTTGAAGGAAATG-3′	Construction of pEX19Gm *pctB-wspA*	This study
CHP215	5′-TTGGGGAAAAATGTGCTTCGCTCGCTGTCGTTTGCCAAG-3′	Construction of pEX19Gm *pctC-wspA*	This study
CHP216	5′-CAGGCCCTTGAGCTCCTCGACGAAGCGGTTGAAGGCATT-3′	Construction of pEX19Gm *pctC-wspA*	This study
CHP244	5′-GCCACCGCCACCGCCGATCTTGAAGCTGTCCACCA-3′	Construction of pEX19Gm *pctA-yfp* and pEX18Gm *wspA-pctA-yfp*	This study
CHP245	5′-GACGAGCTGTACAAGTGAGACCGGCCGGTCTCGTCGCC-3′	Construction of pEX19Gm *pctA-yfp*	This study
CHP246	5′-GGCCAGTGCCAAGCTTTCGAAGTGATCAAGGGCATCTCCG-3′	Construction of pEX19Gm *pctA-yfp* and pEX18Gm *wspA-pctA-yfp*	This study
CHP247	5′-CGGTACCCGGGGATCCTCCAGCAGGGCCTGGGTGGTCTCC-3′	Construction of pEX19Gm *pctA-yfp*	This study
CHP248	5′-GGCCAGTGCCAAGCTTAGCGCCTGGACCAGCAGATCGCCT-3′	Construction of pEX18Gm *wspA-pctA*	This study
CHP249	5′-CGGTACCCGGGGATCCCACTCCGGCGGGAACAGCAGGGCG-3′	Construction of pEX18Gm *wspA-pctA* and pEX18Gm *wspA-pctA-yfp*	This study
CHP250	5′-TTCGGCCATGCCGTTGAAACCGGTC-3′	Construction of pEX18Gm *wspA-pctA*	This study
CHP251	5′-AACGGCATGGCCGAACGTATCCATCGCTCGATCCGGGAA-3′	Construction of pEX18Gm *wspA-pctA*	This study
CHP252	5′-GACACGGTCACAGCCTCAGATCTTGAAGCTGTCCACCA-3′	Construction of pEX18Gm *wspA-pctA*	This study
CHP253	5′-GGCTGTGACCGTGTCCCGCTCCACCG-3′	Construction of pEX18Gm *wspA-pctA*	This study
CHP270	5′-GACGAGCTGTACAAGTGAGGCTGTGACCGTGTCCCGCTCCA-3′	Construction of pEX18Gm *wspA-pctA-yfp*	This study
CHP292	5′-GCCACCGCCACCGCCGACTTTGAAGCGGGATACGCCGTT-3′	Construction of MINICTX2-*wspA-yfp* and MINICTX2-*wspA*-5-*yfp*	This study
CHP303	5′-AATTTGGATCCCGTGTTCCGCCGGACCAGCGAG-3′	Construction of pEX19GmΔ*wspD*	This study
CHP304	5′-TTAAAAGCTTCGCTTCCGGCAGACCGGGAGTCG-3′	Construction of pEX19GmΔ*wspD*	This study
CHP322	5′-AGTGAACAAAGATGGTTGAGGCGAGCGAAGGCCCTCTCCTGCAAGCCGTA-3′	Construction of pEX19GmΔ*wspD*	This study
CHP323	5′-TACGGCTTGCAGGAGAGGGCCTTCGCTCGCCTCAACCATCTTTGTTCACT-3′	Construction of pEX19GmΔ*wspD*	This study
CHP334	5′-AATTGGTACCTCAGACTTTGAAGCGGGATACGCCGT-3′	Construction of MINICTX2-*wspA,* MINICTX2-*wspA*-5-*yfp* and MINICTX2-*wspA*-5	This study
CHP335	5′-TATAGGGCGAATTGGGTACCTTACTTGTACAGCTCGTCCATGCC-3′	Construction of MINICTX2-*wspA-yfp* and MINICTX2-*wspA*-5-*yfp*	This study
CHP336	5′-	Construction of MINICTX2-*wspA-yfp*, MINICTX2-*wspA*,	This study

	TAGAACTAGTGGATCCAATCGCGCCCCGT TCCCGGCAT-3′	MINICTX2-*wspA*-5-*yfp* and MINICTX2-*wspA*-5	
CHP337	5′-GACCAACCTGCTCGCCCTGAACGCCGCCA TCGA-3′	Construction of MINICTX2-*wspA*-5 -*yfp*	This study
CHP338	5′-TCGATGGCGGCGTTCAGGGCGAGCAGGTT GGTC-3′	Construction of MINICTX2-*wspA*-5 -*yfp*	This study
CHP339	5′-AATTTGGTACCGGTCACCACCATCGTCAAG GTCGCC-3′	Construction of pEX19Gm Δ*wspB* (for *wspB* deletion in a background with WT *wspA*)	This study
CHP340	5′-AATTAAGCTTCCCCAGTTCGTCGCCGCGGA AGGAAT-3′	Construction of pEX19Gm Δ*wspB* (for *wspB* deletion in a background with WT *wspA*) and pEX19Gm Δ*wspB* (for *wspB* deletion in a background with *wspA*-*yfp*)	This study
CHP341	5′-GCTTCAAAGTCTGAGGCTGTGACCCCGGA GTGCGGCGAGGGGACGGCA-3′	Construction of pEX19Gm Δ*wspB* (for *wspB* deletion in a background with WT *wspA*)	This study
CHP342	5′-TGCCGTCCCCTCGCCGCACTCCGGGGTCAC AGCCTCAGACTTTGAAGC-3′	Construction of pEX19Gm Δ*wspB* (for *wspB* deletion in a background with WT *wspA*)	This study
pctB_F	5′-GCCATGGAATTCATGATCAAAAGTCTCAA GTTCAGCCACAAG-3′	Construction of pEX19Gm-*pctB*-*yfp*	This study
pctB_R	5′-GCTATCGGTACCGGGATCTTGAAGCTGTC CACCAGCTG-3′	Construction of pEX19Gm-*pctB*-*yfp*	This study
pctBdown_F	5′-CGATAGTCTAGAGCACTGCCCGAGCCGTA GCCGAACCGGCGGATAATC-3′	Construction of pEX19Gm-*pctB*-*yfp*	This study
pctBdown_R	5′-GCCACAAAGCTTTCAGCCATAGCGCGGGA ACCTCGCCTCGCT-3′	Construction of pEX19Gm-*pctB*-*yfp*	This study
WspB-YFP-upR	5′-GCAGGCGTTCGAAGCGATCGTTCATGCGG TCACAGCCTCTAGAGTCGCG-3′	Construction of pEX19Gm Δ*wspB* (for *wspB* deletion in a background with *wspA*-*yfp*)	(Guvener & Harwood, 2007)
WspBdownF	5′-CGCGACTCTAGAGGCTGTGACCGCATGAA CGATCGCTTCGAACGCCTGC-3′	pEX19Gm Δ*wspB* (for *wspB* deletion in a background with *wspA*-*yfp*)	(Guvener & Harwood, 2007)
wspF reverse	5′-NNGGATCCCAGTGAGCGACGCACAGCCTC TCC-3′	Construction of pEX18Gm*wspE*ΔREC in Δ*wspF* background	This study/(Hickman et al., 2005)

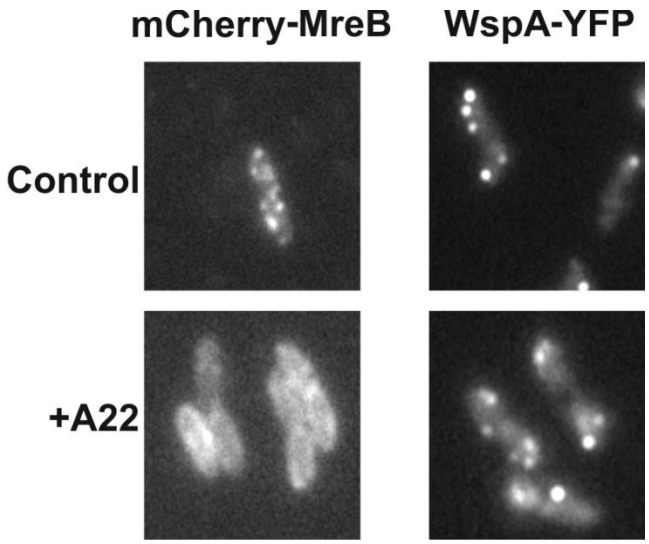

Figure S1: Effect of A22 on MreB and WspA. Cells were exposed to A22 prior to imaging. A22 disrupts MreB localization but has no effect on WspA localization.

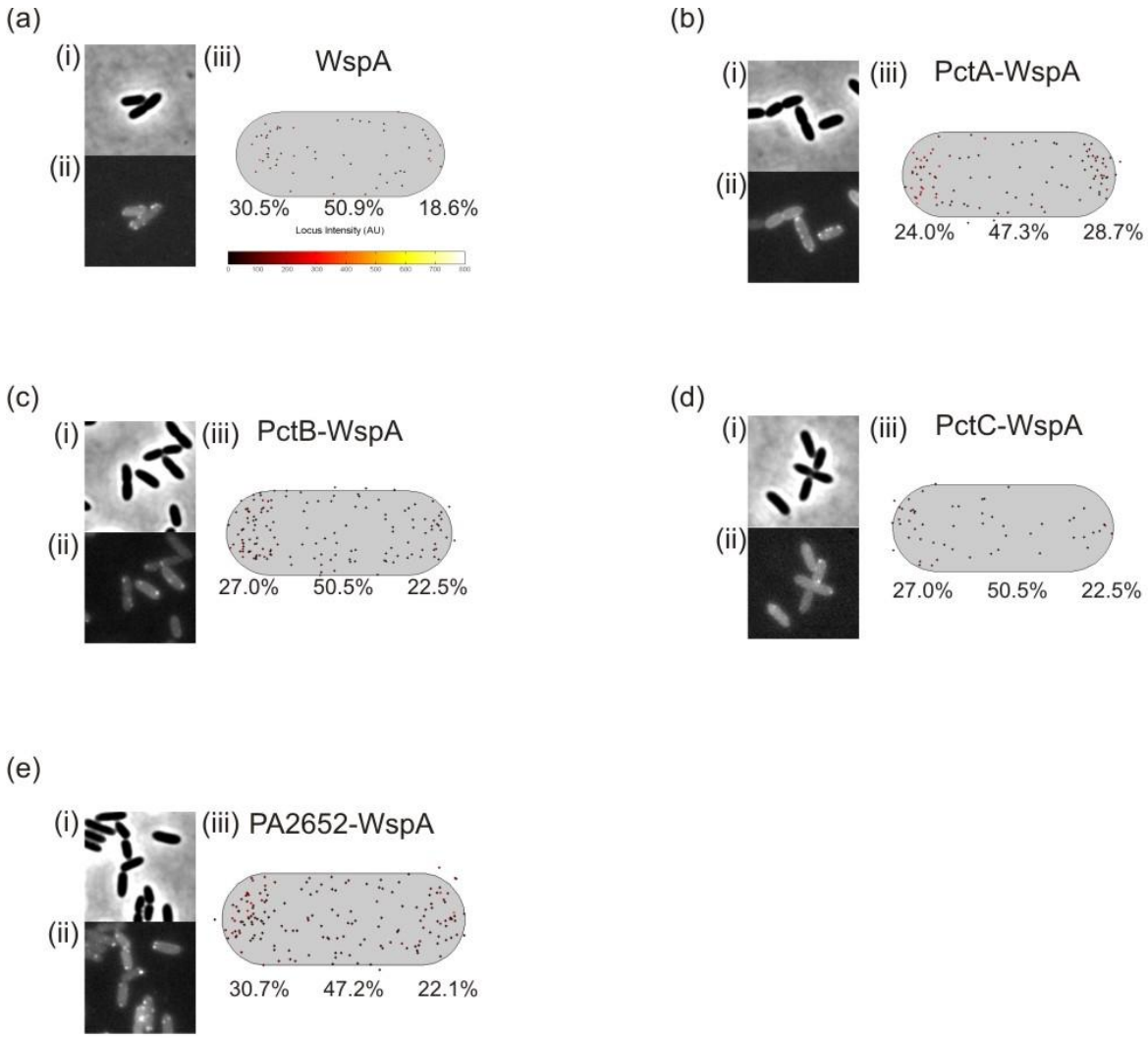

Figure S2: Subcellular localization of chemoreceptor-WspA chimeras. (A) wild-type WspA, (B)PctA-WspA, (C) PctB-WspA, (D) PctC-WspA, (E) PA2652-WspA. (i) Phase contrast, (ii) CFP (iii) Composite data showing the subcellular localization of clusters in a population of cells. The cell length and width represents the average cell size, the cluster intensity is represented by the locus intensity heat map, the brightest cluster in each cell was plotted. The proportion (%) of clusters found in the old (left) or new (right) cell pole or laterally localized is shown.

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(A)

aatcgcgccccgttcccggcatggggacggggcgcacattccctccgtgatgagcctgtcttctcagaaaagtctggcttgccagttttctttctttctctttctgccacacttctggcgtcaaaaaacgcctttccagcgcctacaatagcgggaaaaccctatggcgctgcttgccggtgccggggacg

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gcaagtacctcaacgagatgatcgagctgaacaaggacatcgccgatcgcgcctcggacaacatcgtaaacgcggtggacgatgccgaactgagcatgctggtgaccctgctgctggcggtggtggtagccggcatctgcggtttcctgctgttgcgggcgatcacccagccgatccagaagatcg

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caagcagcagcaggccaccgccaccgaaaccgccgcgaccaccacggagatcggcgccacttctcgcgagatcgccgccacctcgcgcgacctggtgcggaccatgagcgaagtctccggcgccgccgagcagacgtcgac*ccctggccggttccggccagttgggcctggcgcgcatggagg*

*aaaccatgcaccacgtgatgggcgcggccgacctggtcaacgccaagctggcgatcctcaacgagaaggccggcaacatcaaccaggtggtcaccaccatcgtcaaggtcgccgaccagaccaacctgctc**gcc**ctgaacgccgccatcgaggcg**gcg**aaagccggcgagtacggc*

*cgcgggttcgccgtggtggcc**gac**gaggtgcgc**gac**ctggcggac**cgc**accgcggtcgccacctacgacatcgagcagatggtgcgcgagatccagtcggcggtatcggccggggtgatgggcatggacaagttctccgaggaggtccgccgcggtatcgccgaggtcggccaggt*

cggcgagcaactgtcgcagatcatccagcaggtgcaggcgctggcgccgcgggtgcagatggtcaacgagggtatgcaggcccaggctaccggtgccgagcagatcaaccaggcgctggtgcaactgggcgaggccaccgggcagaccgtggagtcgctgcgccaggccagcttc

gccatcgatgagctgaacctggtggcgaacgggctgcgcaacggcgtatcccgcttcaaagtctga

(B)

MKNWTVRQRILASFAVIIAIMLLMAATAYVKMLTVEKGAYRVQDDAMPGMYFITLVRSSWTDNYLQTQELFGITDDHELSKAEADSILASEERLDQQIASYQKTMNPDEARDHELLAGFQAVRKDYLEQHDKVLELYREKRFEEAGKLVA

GPLTEHWREGRKYLNEMIELNKDIADRASDNIVNAVDDAELSMLVTLLLAVVVAGICGFLLLRAITQPIQKIVRSLDLMAGGDLTARLNLGRRDEFGAIETGFNGMAEELKGLVSQAQRSSVQVTTSVTEIAATSKQQQATATETAATTTEI

GATSREIAATSRDLVRTMSEVSGAAEQTSTLAGSGQLGLARMEETMHHVMGAADLVNAKLAILNEKAGNINQVVTTIVKVADQTNLL**S**ALNAAIEA**E**AKAGEYGRGFAVVA**T**D**R**VR**Q**NLADRTAVATYDIEQMVREIQSAVSAGVMGMDKF

SEEVRRGIAEVGQVGEQLSQIIQQVQALAPRVQMVNEGMQAQATGAEQINQALVQLGEATGQTVESLRQASFAIDELNLVANGLRNGVSRFKV

Figure S3: WspA-5 construction. (A) Nucleotide sequence. Entire fragment cloned into MINICTX2 is shown, ORF in blue. The synthetic DNA region cloned into pUC57-3' *wspA*-4 mutations is shown in italics. The arrow denotes the 5th mutation, which was introduced later by SOE PCR (B) Amino acid sequence of WspA-5. Red, underlined codons/amino acids show the locations of the mutations, the WT codon/amino acid is shown in bold, black text above.

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