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Table S1. Regulation of the *cepR2 and cepS* promoters.

Fusion[a]	Fragment[b]	Chromosomal genotype[c]	OHL (uM)	β-Galactosidase	Induction Ratio
P*cepR2-lacZ*	-195 - +170	WT	0	237 ± 8	(1)
		WT	1	480 ± 30	2.0
		GR141 (*cepR2*$^-$)	0	247 ± 23	1.0
		GR141 (*cepR2*$^-$)	1	446 ± 26	1.9
		GR145 (*cepS*$^-$)	0	116 ±13	0.5
		GR145 (*cepS*$^-$)	1	126 ± 12	0.5
P*cepS-lacZ*	-198 - +127	WT	0	264 ± 23	(1)
		WT	1	468 ± 35	1.8
		GR141 (*cepR2*$^-$)	0	237 ± 17	0.9
		GR141 (*cepR2*$^-$)	1	482 ± 32	1.8
		GR145 (*cepS*$^-$)	0	172 ± 14	0.7
		GR145 (*cepS*$^-$)	1	155 ± 21	0.6

a: The *cepR2-lacZ* fusion was carried by plasmid pGR141, while the *cepS-lacZ* fusion was carried by pGR146.

b: Coordinates of the two fusions are calculated relative to the *cepR2* and *cepS* translation start sites, respectively.

c: All strains are derivatives of K56-I2, and therefore have mutations in *cepI*.

Table S2. Bacterial strains used in this study.

Strain	Description	Source and/or reference
B. cenocepacia		
K56-I2	*B. cenocepacia* K56-2 *cepI*::TpR	(Lewenza *et al.*, 1999)
GR141	K56-I2, Δ*cepR2*, *cepI*::TpR	This study
GR145	K56-I2, Δ*cepS*, *cepI*::TpR	This study
E. coli		
DH5α	*F- φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rk-, mk+) phoA supE44 λ-thi-1 gyrA96 relA1*	Stratagene
BL21(DE3)	P*lac*-gene 1 of bacteriophage T7; pTet-TVMV protease, Km^R	(Studier *et al.*, 1990)
MC4100	F- a*raD139* Δ(*argF-lac*)U169 *rpsL150 relA1 deoC1 rbsR fthD5301 fruA*25 λ-	(Ferenci *et al.*, 2009)
SM10(λ*pir)*	*λpir,* RP4 *tra* regulon, host for *pir*-dependent plasmids; Kan^R	(Donnenberg & Kaper, 1991)
EPMax10B	F−*mcrA* Δ(*mrr-hsdRMS-mcrBC*) 80*dlacZ*ΔM15 Δ*lac*X74 *deoR recA1 endA1 araD139* Δ(*ara, leu*)7697 *galU galK rpsL nupG λ*−	(Barrett *et al.*, 2008)
Agrobacterium tumefaciens		
WCF47(pCF218) (pCF372)	R10 Δ*traI;* P*tetR-traR*, P*traI-lacZ* fusion; Tet^R, Sp^R	(Fuqua & Winans 1994)

Table S3. Plasmids used in this study.

Plasmid	Description	Source and/or reference
pRSETa	T7 promoter cloning vector, ColE1 ori, Ap^R	Invitrogen
pT7-*groESL*	PT7-*groESL*, ColE1; Cm^R	(Yasukawa *et al.*, 1995)
pYWN302	Broad host range, promoterless transcriptional *lacZYA* reporter, Tet^R	(Wei *et al.*, 2011)
pSRKGm	pBBR1MCS-2-derived broad-host range expression vector containing *lac* promoter; Gm	(Khan *et al.*, 2008)
pSRKKm	pBBR1MCS-2-derived broad-host range expression vector containing *lac* promoter; Kan^R	(Khan et al., 2008)
pEX18Tet-*pheS*	Suicide plasmid for gene replacement based on *pheS* and Tet^R	(Barrett et al., 2008)
pSW208	pACYC184-derived plasmid containing *lac* promoter; Cm^R	Lab collection
pGR107	*cepR2* cloned into *NdeI* and *XhoI* sites of pRSETa using oligonucleotides GR295 and GR288.	This study
pGR132	PCR fragment using oligonucleotides GR280 and GR284 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion.	This study
pGR133	PCR fragment using oligonucleotides GR281 and GR284 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion.	This study
pGR134	PCR fragment using oligonucleotides GR279 and GR284 cloned into pYWN302 KpnI and XbaI sites; *bcam0191-lacZ* fusion	This study
pGR136	PCR fragment using oligonucleotides GR196 and GR191 cloned into pYWN302 at KpnI and XbaI sites; *bcam0192-lacZ* fusion.	This study
pGR137	PCR fragment using oligonucleotides GR195 and GR191 cloned into pYWN302 at KpnI and XbaI sites; *bcam0192-lacZ* fusion	This study
pGR138	PCR fragment using oligonucleotides GR194 and GR191 cloned into pYWN302 at KpnI and XbaI sites; *bcam0192-lacZ* fusion	This study
pGR139	PCR fragment using oligonucleotides GR193 and GR191 cloned into pYWN302 at KpnI and XbaI sites; *bcam0192-lacZ* fusion	This study
pGR140	PCR fragment using oligonucleotides GR192 and GR191 cloned into pYWN302 at KpnI and XbaI sites; *bcam0192-lacZ* fusion.	This study
pGR141	PCR fragment using oligonucleotides GR197 and GR202 cloned into pYWN302 at KpnI and XbaI sites; *cepR2-lacZ* fusion.	This study
pGR146	PCR fragment using oligonucleotides GR203 and GR208 cloned into pYWN302 at KpnI and XbaI sites; *cepS-lacZ* fusion.	This study
pGR178	PCR fragment made using GR329, GR330, GR331, and GR332, cloned into pEX18Tet-*pheS*; *cepR2* deletion with flanking DNA	This study
pGR182	PCR fragment made using GR345, GR346, GR347, and GR348 cloned into pEX18Tet-*pheS*; *cepS* deletion with flanking DNA.	This study
pGR192	pSRKKm derivative containing *cepR2* using GR288 and GR295.	This study
pGR193	pSRKGm derivative containing *cepS* using GR322 and GR323	This study

pGR195	PCR fragment using oligonucleotides GR292 and GR284 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion	This study
pGR197	PCR fragment using oligonucleotides GR293 and GR284 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion	This study
pGR198	PCR fragment using oligonucleotides GR294 and GR284 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion	This study
pGR236	PCR fragment using oligonucleotides GR275 and GR283 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion	This study
pGR243	PCR fragment using oligonucleotides GR196 and GR380 cloned into pYWN302 at KpnI and XbaI sites; *bcam0192-lacZ* fusion	This study
pGR259	PCR fragment using oligonucleotides GR392 and GR284 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion with altered CepR2 binding site	This study
pGR260	PCR fragment using oligonucleotides GR393 and GR284 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion with altered CepR2 binding site	This study
pGR261	PCR fragment using oligonucleotides GR394 and GR284 cloned into pYWN302 at KpnI and XbaI sites; *bcam0191-lacZ* fusion with altered CepR2 binding site	This study
pGR276	pSW208 derivative containing *cepS* using GR323 and GR417	This study

Table S4. Oligonucleotides used in this study.

Name	Sequence	Comments
GR191	ATTTCTAGACGAATACGTGCCATTCCATG	For PCR amplifying *bcam0192* promoters in pGR136, pGR137, pGR138, pGR139, and pGR140 and Fragment 3.
GR192	ATTGGTACCCTGAAATTGCTGC	For PCR amplifying of *bcam0192* promoter in pGR140.
GR193	ATTGGTACCTGGATGGATGAGGAGTCTG	For PCR amplifying Fragment 3 and pGR139
GR194	ATTGGTACCTTGCGGATGTCAATTCC	For PCR amplifying of *bcam0192* promoter in pGR138.
GR195	ATTGGTACCTCGCATCGTGCATTTC	For PCR amplifying of *bcam0192* promoter in pGR137.
GR196	ATTGGTACCTCACGTCGTTTCTCCTG	For PCR amplifying *bcam0192* promoter in pGR136 and pGR243
GR197	ATTTCTAGACGACGATCTGCATGTCG	For PCR amplifying *cepR2* promoter in pGR141
GR202	ATTGGTACCGTCGATCAGTCCTGATAC	For PCR amplifying *cepR2* promoter in pGR141
GR203	ATTGGTACCCATACTCGTGAGCAAGC	For PCR amplifying *cepS* promoter in pGR146
GR208	ATTTCTAGAATCGGTGATCCTCG	For PCR amplifying *cepS* promoter in pGR146
GR275	GCTTCTAGAGGTGGATGAATTAAATGC	For PCR amplifying *bcam0191* promoter in pGR236
GR279	ATTGGTACCATGAAATGCACGATGCG	For PCR amplifying *bcam0191* promoter in pGR134
GR280	ATTGGTACCCCTCATCCATCCATCAA	For PCR amplifying *bcam0191* promoter in pGR132
GR281	ATTGGTACCATCGGGCTGTCTAGGATAAG	For PCR amplifying *bcam0191* promoter in pGR133
GR283	ATTGGTACCGGACTCTCCTAGTAATGTCC	For PCR amplifying *bcam0191* promoter in pGR130, pGR236.
GR284	ATTTCTAGAGCGTAGATATGCGTCGAC	For PCR amplifying *bcam0191* promoters in pGR130, pGR132, pGR133, pGR134, pGR195, pGR197, pGR198, pGR259, pG260, pGR261 and Fragment 1.
GR288	ATTCTCGAGTTTTTCACGTCACGG	For amplifying *cepR2* for cloning into pGR107 and pGR192
GR292	ATTGGTACCTACTGGAAAGGCTTTGACGCA	For PCR amplifying *bcam0191* promoter in pGR195
GR293	ATTGGTACCCGCACGGAATTGACATC	For PCR amplifying *bcam0191* promoter in pGR197
GR294	ATTGGTACCCCGCAAATCGGGCTGTC	For PCR amplifying *bcam0191* promoter in pGR198
GR295	GGAATTCCATATGGACCTGACAATACTG	For amplifying of *cepR2* for cloning into pGR192 and pGR107
GR301	CCTCTTCGCTATTACGCCAGC	For PCR amplifying of *lacZ* fragment for negative control for EMSA experiments
GR302	CGTTACCCAACTTAATCG	For PCR amplifying of *lacZ* fragment for negative control for EMSA experiments
GR322	GGAATTCCATATGACCAGCGTTCAAGACG	For PCR amplifying *cepS* for cloning into pGR193
GR323	CCCAAGCTTGATCGTGCATGCGATC	For PCR amplifying *cepS* for cloning into pGR193 and pGR276.
GR324	GATCGTGCATGCGATC	For sequence verification of *cepR2* deletion
GR328	GCATGCGTCTTGTTCATCGC	For sequence verification of *cepS* deletion

GR329	ACTAAGCTTCTGCAGTCGCTCGCACAGCTT	With GR330, GR331, and GR332, for construction of *cepR2* deletion fragment cloned into pGR178.
GR330	ATTGAATTCTTGCAGTATTGTCAGGTCCATC	With GR329, GR331, and GR332, for construction of *cepR2* deletion fragment cloned into pGR178.
GR331	ATTGAATTCTCGACGCGATGAACAAGAC	With GR329, GR330, and GR332, for construction of *cepR2* deletion fragment cloned into pGR178.
GR332	ACTTCTAGAGCACGTACGATTCGATCATTCGC	With GR329, GR330, and GR331, for construction of *cepR2* deletion fragment cloned into pGR178.
GR345	ATGGATCCGAACGCATTCCATACGACC	With GR346, GR347, and GR348, for construction of *cepS* deletion fragment cloned into pGR182.
GR346	GCACTAGTGATCAGTCCTGATACGAAACCG	With GR345, GR347, and GR348, for construction of *cepS* deletion fragment cloned into pGR182..
GR347	GCACTAGTGATCGCATGCACGATCCG	With GR345, GR346, and GR348, for construction of *cepS* deletion fragment cloned into pGR182..
GR348	CGGAATTCGACATCATGTGCTTGGC	With GR345, GR346, and GR347, for construction of *cepS* deletion fragment cloned into pGR182..
GR351	ATTTCTAGACATATTCGCATCGTGCATTTC	For PCR amplifying Fragments 2, 4, and 5
GR365	ATTTCTAGAGGATAAGAATTGCGATTCATC	For PCR amplifying Fragment 1
GR380	ATTTCTAGATTCCGGCGCCGGAAACCGTTT	For PCR amplification of *bcam0192* promoter in pGR243
GR391	ATGGTACCCGCACGGAATTGACATCCGCAA ATCGGGCTGTC	For PCR amplifying Fragment 2
GR392	ATGGTACCCGCACGGAATTCTGATCCGCAA ATCGGGCTGTC	For PCR amplification of bcam0191 promoter in pGR259 and Fragment 4
GR393	ATGGTACCCGCACGGAATTGACTAGGGCAAA TCGGGCTGTC	For PCR amplification of bcam0191 promoter in pGR260 and Fragment 5
GR394	ATGGTACCCGCACGGAATTGACATCCCGTT ATCGGGCTGTC	For PCR amplification of bcam0191 promoter in pGR261
GR417	ATGAATTCAGGAGGCGATAGATGACCAGCG TTCAAGA	For PCR amplifying *cepS* and cloning into pGR276
GR458	6-FAM-ATCGGTCACGTCGTTTCTCC	For mapping *bcam0191* promoter and DNA footprinting analysis
GR459	6-FAM-AATTGCCTGCATGCCGT	For mapping *bcam0192* promoter

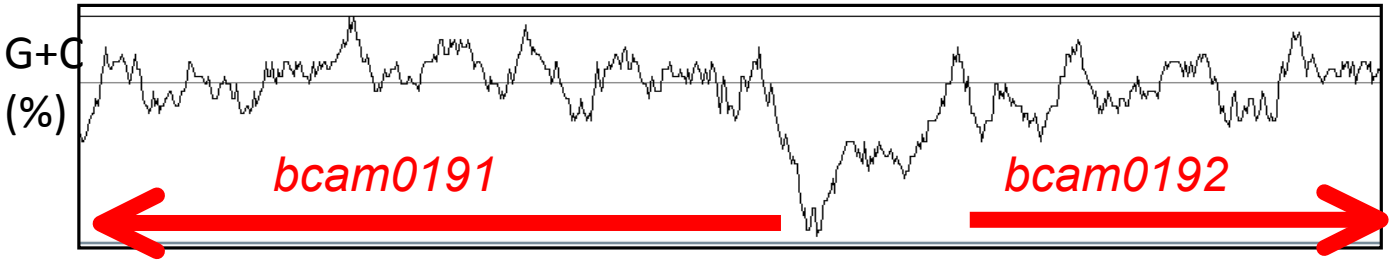

Fig. S1. G/C content of *bcam0191*, *bcam0192*, and the intergenic region between them, using a sliding window of 15 nucleotides. The intergenic region contains a significantly A/T-rich sequence.

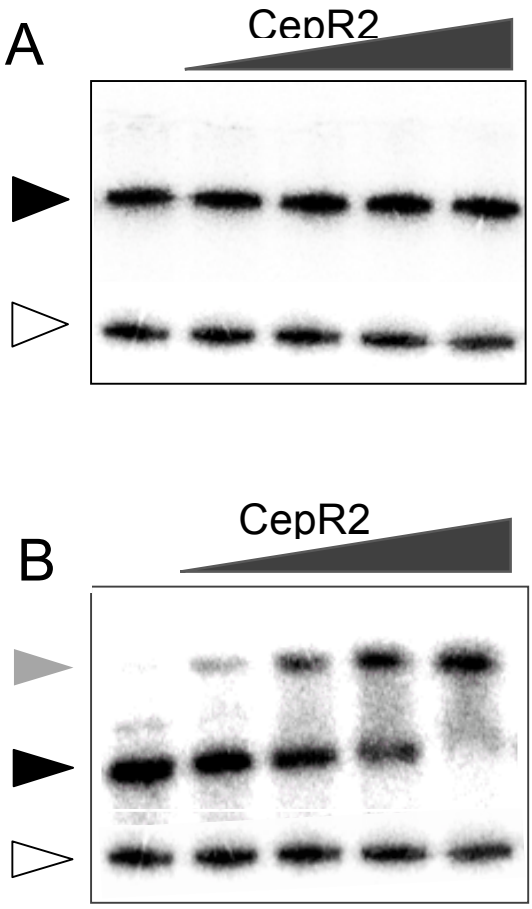

Fig. S2. CepR2 does not detectably shift a DNA fragment containing the *cepR2-cepS* intergenic region. A: A 197 nucleotide DNA fragment containing the entire *cepR2-cepS* intergenic region. B: DNA fragment containing the *bcam0191-bcam0192* intergenic region (see Fig. 6, fragment 2). Filled arrowheads show free DNA. Grey arrowhead shows CepR2-DNA complex. Open arrowheads show a negative control fragment containing *lacZ* DNA.

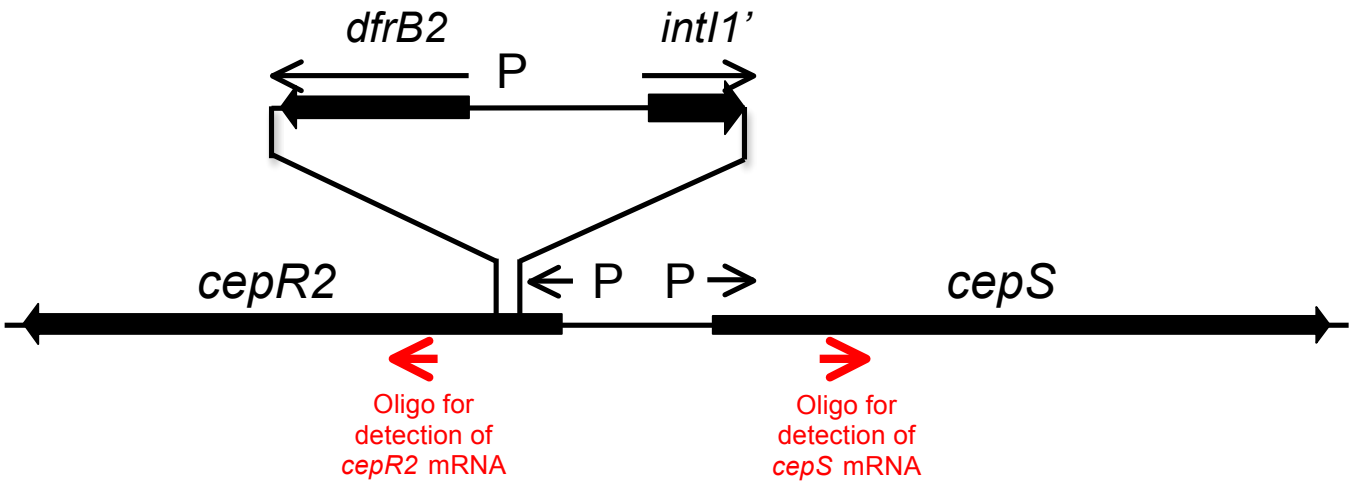

Fig. S3. In the study of Malott and colleagues, an approximately 620-nucleotide fragment containing *dfrB2* (trimethoprim resistance) and *intI1'* (5' end of the intI1 integrase gene of plasmid R388) was inserted into a gap in *cepR2* created by digestion with SacII (which digests sites at two sites 54 and 87 nucleotides downstream from the *cepR2* start codon (Malott *et al.*, 2009). The orientation of the insertion was not described. Divergent promoters in this cassette (DeShazer & Woods, 1996) could have caused elevated expression of *cepS* gene and of that portion of *cepR2* downstream of the insertion.

CepR2 mRNA was detected using oligonucleotide 5'–CGGCAATCATCCACGCGAATGGGAACACCGTTATGTGAAATTCGGCTACGTCACCATCGA–3', which is located 167-227 downstream of the *cepR2* translation start site.

CepS mRNA was detected using oligonucleotide 5'–GGCCGGGCAACTATTGTTTACGACGCGCGGCGTATTAGTACTCGACGTCGAAGGCCAGCG–3', which is located 142-203 nucleotides downstream of the *cepS* translation start site.

SUPPLEMENTARY MATERIAL

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