Supplementary Materials

Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Genotype and description[a]	Source/reference
***Pseudomonas pseudoalcaligenes* CECT5344**		
Wild-type	Nx^R	[1]
$CynS^-$	*cynS* mutant; Gm^R	[2]
$CynF^-$	*cynF* mutant; Km^R	This work
$CynBD^-$	*cynBD* mutant; Gm^R	This work
$CynX^-$	*cynX* mutant; Km^R	This work
$CynX^-$/$CynBD^-$	*cynX* and *cynBD* double mutant; Km^R and Gm^R	This work
Escherichia coli		
DH5α	*lac*$^-$, host for plasmids carrying *lacZ*	[3]
S17-1	*tra*$^+$, host for the mobilizable mob plasmids	[4]
Plasmids		
pBluescript KS (+)	Ap^R, cloning vector	Stratagene
pBmod	Ap^R, pBluescript Δ*Sal*I (deleted between *Eco*RV and *Hin*dII sites)	This work
pGEM-T Easy	Ap^R, cloning vector	Promega
pK18mob	Km^R, mobilizable suicide vector in *P. pseudoalcaligenes*, derived from pK18	[5]
pGEMT-*cynF*	pGEM-T Easy containing *cyn*F	This work
pBmod-*cynF*	pBmod containing *cynF*	This work
pBmod-*cynF::Km*	pBmod with *cynF::Km* insertion	This work
pK18mob-*cynF::Km*	pK18mob with *cynF::Km* insertion	This work
pGEMT-*cynB*	pGEM-T Easy containing *cynB*	This work
pK18mob-*cynD*	pK18mob containing *cynD*	This work
pK18mob-*cynBD*	pK18mob containing *cynB* and *cynD*	This work
pK18mob-*cynBD::Gm*	pK18mob with *cynBD::Gm* insertion	This work
pK18mob-*cynX*	pK18mob containing *cynX*	This work

[a]Abbreviations: Ap^R, ampicillin resistant; Gm^R, gentamicin resistant; Km^R, kanamycin resistant; Nx^R, nalidixic acid resistant.

Table S2. Oligonucleotides used in this work.

Primer	Sequence (5'→ 3')[a]	Used for
CynLF10	TCTCTGGGCGCATGACCTTGGGGCAC	*cynF* mutagenesis
CynLR7	CGGCTCGCGCAAGGAAGTGAAGAACG	*cynF* mutagenesis
CynLR9	TCTCCGCGCTGCTGTTGAAGCTTCTA (*Hin*dIII)	*cynBD* mutagenesis
CynLF11	CAACGTGGATCCAGTCGCGGCGCAC (*Bam*HI)	*cynBD* mutagenesis
CynLR8	CGGATCCGGCAAGTCGACCATTCTCA (*Bam*HI)	*cynBD* mutagenesis
CynLF8	CGGGCCGTTGGTCATCAGCAGAATTC (*Eco*RI)	*cynBD* mutagenesis
3113FB	CGGGATCCGGCAGTGCCATCGCGCTGGGTGCTGCCA (*Bam*HI)	*cynX* mutagenesis
3113RH	CGAAGCTTGCAGGTGACCAGCCCGCACGCCAGCAACA (*Hin*dIII)	*cynX* mutagenesis
Cyn1R	ATGCAGGATGCGGTCGTGGATTGGAT	RT-PCR
Cyn1F	TGGGAAAGCTCGCTGTCGGGGCTGAA	RT-PCR
Cyn2R	CGATCTCGAGGGGCAACTACCTCAACCAGCCGGTGCC	RT-PCR
Cyn2F	CCATCACGCTCAACGACTTGTAGGCATCCGCCGGTA	RT-PCR
CynBR	GGATTCGCAGACCTCGGCAATCTTCGT	RT-PCR
CynDF	CGGGAAGCGTTTGGCCAGGCGTCGG	RT-PCR
Cyn3R	CGATCCTGCTTTCCGACCGCATTCTGCTGATGACCAACG	RT-PCR
Cyn3F	TAGAAGAGGTAGATCAACGGGTCGGTGGGCACGGCCT	RT-PCR
rpoBF	AGCTGCTGCGTGCGATCTTCGGTGAGA	RT-PCR (housekeeping)
rpoBR	CCAATTGCTCGTTCAGGGCGTCGTCAG	RT-PCR (housekeeping)
qCynSF	TGCTCGAATTGCCGGCGGAGGTCTC	qRT-PCR
qCynSR	GGGTCGGTGGGCACGGCCTTG	qRT-PCR

[a]The sites for the restriction enzymes indicated in brackets are underlined.

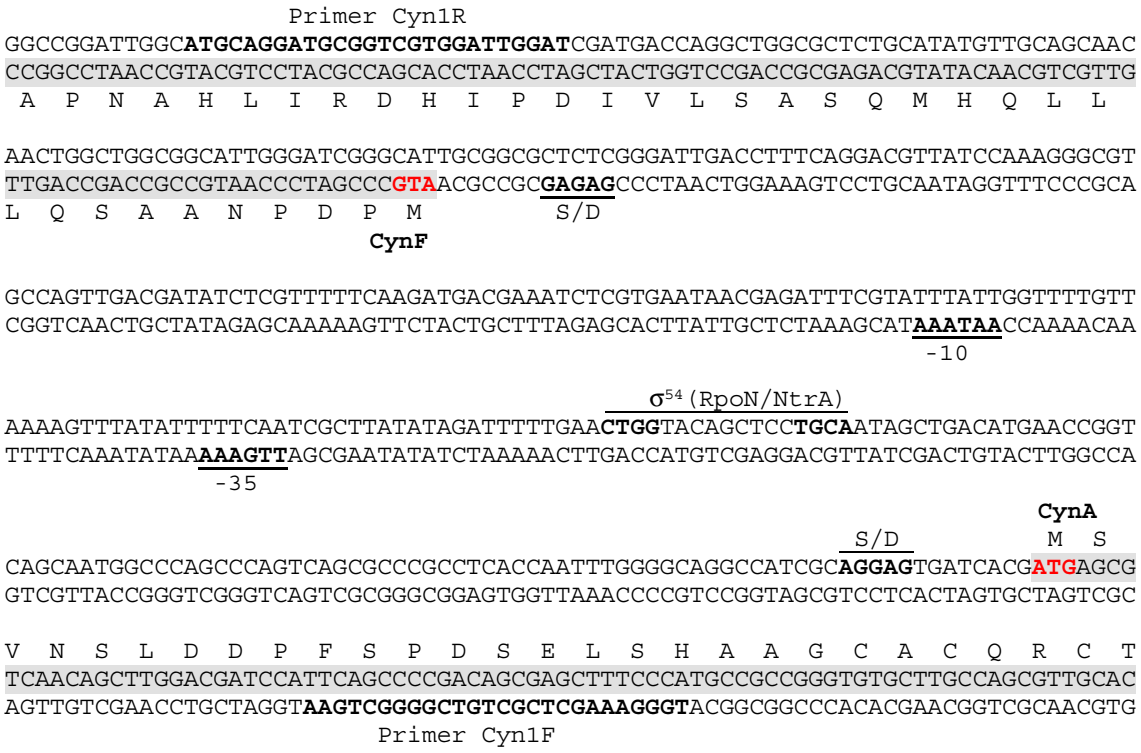

Figure S1. Promoter region of the *cynF* and *cynA* genes of *P. pseudoalcaligenes* CECT5344. The *cynF* and *cynA* genes are divergently transcribed. The ATG start codons for both CynF and CynA proteins are indicated in red color, and their putative Shine-Dalgarno (S/D) sequences for ribosomal binding are shown in bold and underlined. The N-terminal amino acid sequences of CynF and CynA are also presented, with the corresponding DNA coding sequence shaded in grey. The putative σ^{70}-dependent promoter of the *cynF* gene (TATA box at position -10 and its upstream -35 sequence), and the σ^{54}-dependent promoter of the *cynA* gene (RpoN/NtrA binding site) are indicated. Primers Cyn1R and Cyn1F used for the amplification of the intergenic region are also marked.

References

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