

## Supplementary Materials

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

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

<sup>a</sup>Abbreviations: Ap<sup>R</sup>, ampicillin resistant; Gm<sup>R</sup>, gentamicin resistant; Km<sup>R</sup>, kanamycin resistant; Nx<sup>R</sup>, nalidixic acid resistant.

**Table S2.** Oligonucleotides used in this work.

| Primer  | Sequence (5'→ 3') <sup>a</sup>                                     | Used for                 |
|---------|--------------------------------------------------------------------|--------------------------|
| CynLF10 | TCTCTGGGCGCATGACCTTGGGGCAC                                         | <i>cynF</i> mutagenesis  |
| CynLR7  | CGGCTCGCGCAAGGAAGTGAAGAACG                                         | <i>cynF</i> mutagenesis  |
| CynLR9  | TCTCCGCGCTGCTGTTGA <u>AAGCTT</u> CTA ( <i>HindIII</i> )            | <i>cynBD</i> mutagenesis |
| CynLF11 | CAACGT <u>GGATCC</u> AGTCGCGGCGCAC ( <i>Bam</i> HI)                | <i>cynBD</i> mutagenesis |
| CynLR8  | <u>CGGATCC</u> GGCAAGTCGACCATTCTCA ( <i>Bam</i> HI)                | <i>cynBD</i> mutagenesis |
| CynLF8  | CGGGCCGTTGGTCATCAGCAGA <u>AATTC</u> ( <i>Eco</i> RI)               | <i>cynBD</i> mutagenesis |
| 3113FB  | CG <u>GATCC</u> GGCAGTGCCATCGCGCTGGGTGCTGCCA ( <i>Bam</i> HI)      | <i>cynX</i> mutagenesis  |
| 3113RH  | CGA <u>AAGCTT</u> GCAGGTGACCAGCCCGCACGCCAGCAACA ( <i>HindIII</i> ) | <i>cynX</i> mutagenesis  |
| Cyn1R   | ATGCAGGATGCGGTCGTGGATTGGAT                                         | RT-PCR                   |
| Cyn1F   | TGGGAAAGCTCGCTGTCTGGGGCTGAA                                        | RT-PCR                   |
| Cyn2R   | CGATCTCGAGGGGCAACTACCTCAACCAGCCGGTGCC                              | RT-PCR                   |
| Cyn2F   | CCATCACGCTCAACGACTTGTAGGCATCCGCCGGTA                               | RT-PCR                   |
| CynBR   | GGATTGCGAGACCTCGGCAATCTTCGT                                        | RT-PCR                   |
| CynDF   | CGGGAAGCGTTTGGCCAGGCGTCGG                                          | RT-PCR                   |
| Cyn3R   | CGATCCTGCTTTCCGACCGCATTCTGCTGATGACCAACG                            | RT-PCR                   |
| Cyn3F   | TAGAAGAGGTAGATCAACGGGTCGGTGGGCACGGCCT                              | RT-PCR                   |
| rpoBF   | AGCTGCTGCGTGCGATCTTCGGTGAGA                                        | RT-PCR<br>(housekeeping) |
| rpoBR   | CCAATTGCTCGTTCAGGGCGTCGTCAG                                        | RT-PCR<br>(housekeeping) |
| qCynSF  | TGCTCGAATTGCCGGCGGAGGTCTC                                          | qRT-PCR                  |
| qCynSR  | GGGTCGGTGGGCACGGCCTTG                                              | qRT-PCR                  |

<sup>a</sup>The sites for the restriction enzymes indicated in brackets are underlined.

Primer Cyn1R

GGCCGGATTGGC**ATGCAGGATGCGGTCTGGATTGGAT**CGATGACCAGGCTGGCGCTCTGCATATGTTGCAGCAAC  
 CCGGCCTAACCGTACGTCTCTACGCCAGCACCTAACCTAGCTACTGGTCCGACCGGAGACGTATACAACGTCGTTG  
 A P N A H L I R D H I P D I V L S A S Q M H Q L L

AACTGGCTGGCGGCATTGGGATCGGGCATTGCGGCGCTCTCGGGATTGACCTTTTCAGGACGTTATCCAAAGGGCGT  
 TTGACCGACCGCCGTAACCCTAGCCC**GTA**ACGCCGC**GAGAG**CCCTAACTGGAAAGTCCTGCAATAGGTTTCCCGCA  
 L Q S A A N P D P M S/D

**CynF**

GCCAGTTGACGATATCTCGTTTTTCAAGATGACGAAATCTCGTGAATAACGAGATTTTCGTATTTATTGGTTTTTGT  
 CGGTCAACTGCTATAGAGCAAAAAGTTCTACTGCTTTAGAGCACTTATTGCTCTAAAGCAT**AAATAA**CCAAAACAA  
 -10

$\sigma^{54}$  (RpoN/NtrA)

AAAAGTTTATATTTTCAATCGCTTATATAGATTTTTGAAC**CTGGT**TACAGCTCCT**GCA**ATAGCTGACATGAACCGGT  
 TTTTCAAATATA**AAAGTT**AGCGAATATATCTAAAACTTGACCATGTGCGAGGACGTTATCGACTGTACTTGGCCA  
 -35

**CynA**

S/D M S

CAGCAATGGCCCAGCCAGTCAGCGCCCGCCTCACCAATTTGGGGCAGGCCATCGC**AGGAGT**GATCACG**ATG**AGCG  
 GTCGTTACCGGTCGGGTGTCGCGGGCGGAGTGTTAAACCCCGTCCGGTAGCGTCCTCACTAGTGCTAGTCGC

V N S L D D P F S P D S E L S H A A G C A C Q R C T  
 TCAACAGCTTGGACGATCCATTGAGCCCCGACAGCGAGCTTTCCCATGCGCGCGGTGTGCTTGCCAGCGTTGCAC  
 AGTTGTGCAACCTGCTAGGT**AAGTCGGGGCTGTGCTCGA**AGGGTACGGCGGCCACACGAACGGTCGCAACGTG

Primer Cyn1F

**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  $\sigma^{70}$ -dependent promoter of the *cynF* gene (TATA box at position -10 and its upstream -35 sequence), and the  $\sigma^{54}$ -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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