

Supplementary Materials

The connection between Czc and Cad systems involved in cadmium resistance in *Pseudomonas putida*

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

| Strains and plasmids | Description | Reference |
|----------------------------------|--|----------------|
| Strains | | |
| <i>E. coli</i> S17-1 | <i>RP4</i> , <i>mob</i> ⁺ , <i>λpir</i> , host for plasmid construction | Lab stock |
| <i>E. coli</i> BL21(DE3) | <i>F</i> , <i>ompT</i> , <i>hsdS</i> (<i>rB</i> ⁻ <i>mB</i> ⁻), <i>gal</i> , <i>dcm</i> (DE3), host for protein expression | Vazyme Biotech |
| <i>P. putida</i> KT2440 | Wild-type <i>Pseudomonas putida</i> KT2440 | [1] |
| <i>P. putida</i> Δ <i>czcRS3</i> | <i>czcRS3</i> (PP_1437-1438) deletion mutant of KT2440 | This study |
| <i>P. putida</i> Δ <i>cadR</i> | <i>cadR</i> (PP_5140) deletion mutant of KT2440 | This study |
| Plasmids | | |
| pDS3.0 | Suicide vector, Gm ^R , <i>R6K ori</i> , <i>sacB</i> | [2] |
| pDS- <i>czcRS3</i> | Knockout vector for <i>czcRS3</i> | This study |
| pDS- <i>cadR</i> | Knockout vector for <i>cadR</i> | This study |
| pBBR1-403 | Expression vector, Gm ^R , <i>lacI</i> , <i>tac</i> promoter | [3] |
| pB403- <i>czcRS3</i> | pBBR1-403 carrying complete <i>czcRS3</i> | This study |
| pB403- <i>cadR</i> | pBBR1-403 carrying <i>cadR</i> | This study |
| pBRTZ | Reporter vector, Tet ^R , promoter-less <i>lacZ</i> | [4] |
| pBRTZ- <i>czcD</i> | pBRTZ carrying the promoter of <i>czcD</i> (PP_0026) | This study |
| pBRTZ- <i>cadA1</i> | pBRTZ carrying the promoter of <i>cadA1</i> (PP_0041) | This study |
| pBRTZ- <i>czcC1</i> | pBRTZ carrying the promoter of <i>czcC1</i> (PP_0045) | This study |
| pBRTZ- <i>cadA2</i> | pBRTZ carrying the promoter of <i>cadA2</i> (PP_0586) | This study |
| pBRTZ- <i>czcR3</i> | pBRTZ carrying the promoter of <i>czcR3</i> (PP_1438) | This study |
| pBRTZ- <i>czcC2</i> | pBRTZ carrying the promoter of <i>czcC2</i> (PP_2408) | This study |
| pBRTZ- <i>cadA3</i> | pBRTZ carrying the promoter of <i>cadA3</i> (PP_5139) | This study |
| pBRTZ- <i>czcC</i> | pBRTZ carrying the promoter of <i>czcC</i> (PP_5385) | This study |
| pET28a | Expression vector, Kan ^R , <i>lacI</i> , T7 promoter | Lab stock |
| pET28a- <i>czcR3</i> | pBRTZ carrying the encoding sequence of <i>czcR3</i> | This study |
| pET28a- <i>cadR</i> | pBRTZ carrying the encoding sequence of <i>cadR</i> | This study |

Table S2. The primers used in this study.

| Primers | Sequence (5' to 3') |
|----------------------------------|--|
| Primers for mutant construction | |
| <i>czcRS3upS</i> | AGGTACCGCATGCGATATCGAGCTCATGCAGTCGCTGCTGGTCTT |
| <i>czcRS3upA</i> | CCAGGTCATACGCCTGTTGG |
| <i>czcRS3dnS</i> | CCAACAGGCGTATGACCTGGGCGCTTGTTTCGAGCGGTTTT |
| <i>czcRS3dnA</i> | TTTGTGGAATTCCCGGGAGAGCTCAAGGTGGCCTGGATGTGGCA |
| <i>cadRupS</i> | AGGTACCGCATGCGATATCGAGCTCACGCCAAGCACGCGGTTGAT |
| <i>cadRupA</i> | CATGGGAATGTTTCGGTATCC GCTCGTAGTAGCGGATGGTTTC |
| <i>cadRdnS</i> | CGGATACCGAACATTCCCATG |
| <i>cadRdnA</i> | TTTGTGGAATTCCCGGGAGAGCTCGCACGCCGTTCAACCTCACC |
| Primers for promoter | |
| <i>czcDpS</i> | CACCGCGGTGGCGGCCGCTCTAGAGAGCAATATGATCGCCATGG |
| <i>czcDpA</i> | TGAATGAGATTTAGTCATCTGCAGGCTGCCATGGTCATGATTTG |
| <i>cadA1pS</i> | CACCGCGGTGGCGGCCGCTCTAGATCGTACGTCAGTCGCAGGAC |
| <i>cadA1pA</i> | TGAATGAGATTTAGTCATCTGCAGAGGGCGTTCAAGCAAACCTCT |
| <i>czcC1pS</i> | CACCGCGGTGGCGGCCGCTCTAGA CCGCCAAATACAGTGGTACTT |
| <i>czcC1pA</i> | TGAATGAGATTTAGTCATCTGCAGGACATTGCGGTTATACCGGGG |
| <i>cadA2pS</i> | CACCGCGGTGGCGGCCGCTCTAGACCTCCTCAGGTTTTTCACAA |
| <i>cadA2pA</i> | TGAATGAGATTTAGTCATCTGCAGCAGGTCGTATGTGGTGGATGC |
| <i>czcR3pS</i> | CACCGCGGTGGCGGCCGCTCTAGACCACCTCACGCGCCTTCTC |
| <i>czcR3pA</i> | TGAATGAGATTTAGTCATCTGCAGGGTACGCAGCTCGTCCTCG |
| <i>czcC2pS</i> | CACCGCGGTGGCGGCCGCTCTAGAGAGGGTTTCGTGGCCGTA |
| <i>czcC2pA</i> | TGAATGAGATTTAGTCATCTGCAGGAAACAGTCCTCTGGGCGAA |
| <i>cadA3pS</i> | CACCGCGGTGGCGGCCGCTCTAGAAAGGTCAGCCGCTCCACAT |
| <i>cadA3pA</i> | TGAATGAGATTTAGTCATCTGCAGGTGTTTCGTGGCTGACAGGC |
| <i>czcCpS</i> | CACCGCGGTGGCGGCCGCTCTAGATCGCTTTGGCCTACACTCG |
| <i>czcCpA</i> | TGAATGAGATTTAGTCATCTGCAGCCCTGAATGGGAGCATAGG |
| Primers for gene cloning | |
| <i>czcRS3oeS</i> | ATTTACACAGGAAACAGAATTCATGCGCCTACTGATCATCGAG |
| <i>czcRS3oeA</i> | GATCCGCCAAAACAGCCAAGCTTTTATGCCGATGCCGAAACC |
| <i>cadRoeS</i> | ATTTACACAGGAAACAGAATTCATGAAGATCGGAGAACTGGCC |
| <i>cadRoeA</i> | GATCCGCCAAAACAGCCAAGCTT CCGATGGCGGTCTGATAGATC |
| Primers for protein purification | |
| <i>cadRs</i> | TTTAAGAAGGAGATATACCATGAAGATCGGAGAACTGGCC |
| <i>cadRa</i> | GTGGTGGTGGTGGTGTCTCGAGATGCCCCGTGACTCCGCCCC |
| <i>czcR3s</i> | ACTTTAAGAAGGAGATATACCATGCGCCTACTGATCATCGAG |
| <i>czcR3a</i> | TGGTGGTGGTGGTGGTGTCTCGAGAAGGCGAGCCTCAAGTACGTA |
| Primers for FAM-tagged probe | |
| M13F-fam | TGTAAAACGACGGCCAGT (5' end contains 6-FAM tag) |
| M13F- <i>czcC1pS</i> | TGTAAAACGACGGCCAGT GTATCGAAATCATCGTGTC |
| <i>czcC1pA2</i> | TGCGGTTATACCGGGGCAC |
| M13F- <i>czcC2pS</i> | TGTAAAACGACGGCCAGT GATTATCGAACTTATTGGGC |
| <i>czcC2pA2</i> | AGCATTGGCCAGGGACTGTA |
| M13F- <i>czcR3pS</i> | TGTAAAACGACGGCCAGTTACAGGCGCGGGTTTACTCG |
| <i>czcR3pA2</i> | CATGGGGGGCTCGGTATGT |
| <i>czcR3p-mS</i> | TTGGGGCGCTTGACAAAGATGTCACTACAAAGTCAAGAATCGTC |
| <i>czcR3p-mA</i> | GACGATTCTTGACTTTGTAGTGACATCTTTGTCAAGCGCCCCAA |
| Primers for 5'-RACE | |
| <i>czcRS3-RC</i> | TCGACCACGTAACCGTTCTCGCGAA |
| TSO-DNA | AAGCAGTGGTATCAACGCAGAGTACGCGGG |
| TSO-RNA | AAGCAGUGGUAUCAACGCAGAGUACGCGGG (oligo RNA) |

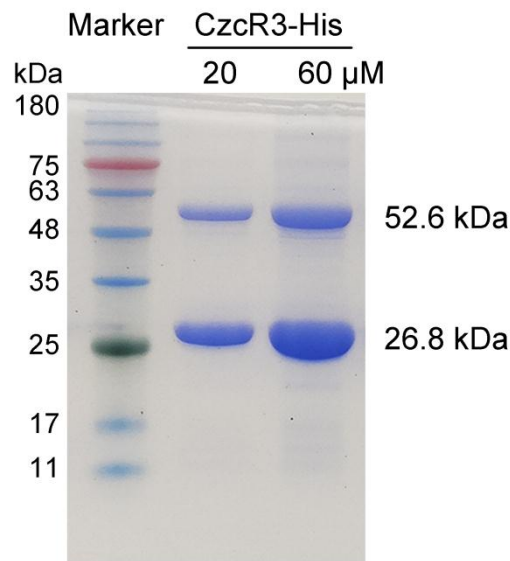


Figure S1. CzcR3 forms a homodimer *in vitro*. The purified His-tagged CzcR3 was diluted to 20 and 60 μM , and then mixed with an equal volume of loading buffer (250 mM Tris-HCl at pH 6.8, 2% SDS, 0.1% bromophenol blue, 20% glycerol, without dithiothreitol (DTT) or β -mercaptoethanol). 20 μl of the sample was electrophoresed in SDS-polyacrylamide gel (SDS-PAGE), and the gel was stained with coomassie brilliant blue. The molecular weight of CzcR3-His monomer is about 26.8 kDa, and that of CzcR3-His homodimer is about 53.6 kDa.

References

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