Supplementary Materials: The Construction of An Engineered Bacterial Strain and Its Application in Accumulating Mercury from Wastewater

Shenghe Chang, Zhengjing Wu, Wei Sun and Haiyan Shu

S1: The sequence of the artificial operon P16S-g10-merT-merP-merB1-merB2-ppk-rpsT.

The sequence of the artificial operon P16S-g10-merT-merP-merB1-merB2-ppk-rpsT. The sequence of P16S is shown in italic capital letters. The 5’ UTR of the g10 gene is shown in bold capital letters. MerT-merP-merB1-merB2-ppk is shown in lower-case letters in sequential order. The initiation codons and termination codons are framed. The sequence of rpsT is shown in capital letters. The restriction enzyme sites used in this study are underlined.

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AATCTCTTTATTTATTTTTCTTCGATATTTTCTTGAATTC

S2: Construction of artificial operons and respective bacterial strains**.**

To differentiate the functions of the genes in the artificial operon, the sequence of linker-P16S-g10 (AATGGGTCGCGGATCCGCTCCCCCGCCGTCGTTCAATGAGAATGGATAAGAGGCTCGTGGGATTGACGTGAGGGGGCAGGGATGGCTATATTTCTGGGAGCGAACTCCGGGCGAATACGAAGCGCTTGGATACAGTTGTAGGGAGGGATTTATCTTTTAACTTTAAGAAGGAG) was synthesized.

PRPSTF and PRPSTR were used to amplify the sequence of rpsT using p5 as a template. The PCR fragment was purified.

PRPSTF: 5'-CGTGTTCTATAACGACAAAACCTCTTATTTTATTG-3'

PRPSTR: 5'-GTGCGGCCGCAAGCTTGAATTCAAGAAAATATCGAA-3'

For constructing the pTPB1B2 plasmid, primers P1 and P2 were used to amplify the gene cluster merT-merP-merB1-merB2 using plasmid p5 as a template. The PCR fragment was purified.

P1: 5'-AACTTTAAGAAGGAGCCAGTATGTCTGAACCACAAAA-3'

P2: 5'-TTGAATTTCGGTTGACCGATCACGGTGTCCTAGATGACAT-3'

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a, the purified merT-merP-merB1-merB2 PCR fragment and the purified rpsT PCR fragment were mixed in a 1:1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pTPB1B2. E. coli BL21 cells harboring the plasmid pTPB1B2 were named BL21-TPB1B2.

For constructing the plasmid pTPK, primers PTPF and PTPR were used to amplify the gene cluster merT-merP using plasmid p5 as a template. The PCR fragment was purified.

PTPF: 5'-AACTTTAAGAAGGAGCCAGTATGTCTGAACCACAAAA-3'

PTPR: 5'-TTGAATTTCGGTTGATATTTGTTGCCTTACTTCTT-3'

PKF and PRPSTR were used to amplify the gene cluster ppk-rpsT using plasmid p5 as a template. The PCR fragment was purified.

PKF: 5'-GAGCTGAAGAAGTAAATGGGTCAGGAAAAGTTATAT-3'

PRPSTR: 5'-GTGCGGCCGCAAGCTTGAATTCAAGAAAATATCGAA-3'

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a, the purified merT-merP PCR fragment and the purified ppk-rpsT PCR fragment were mixed in a 1:1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pTPK. E. coli BL21 cells harboring the plasmid pTPK were named BL21-TPK.

For constructing the plasmid pB1B2K, primers PB1B2KF and PB1B2KR were used to amplify the gene cluster merB1-merB2-ppk using p5 as a template. The PCR fragment was purified.

PB1B2KF: 5’-AACTTTAAGAAGGAGATGGACAAGACTATTTATTCCA-3’

PB1B2KR: 5’-GTGCGGCCGCAAGCTTGAATTCAAGAAAATATCGAA-3’

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a and the purified merB1-merB2-ppk-rpsT PCR fragment were mixed in a 1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pB1B2K. E. coli BL21 cells harboring the plasmid pTPK were named BL21-B1B2K.

For constructing the plasmid pT, primers PTF and PTR were used to amplify the gene merT using p5 as a template. The PCR fragment was purified.

PTF: 5’-AACTTTAAGAAGGAGCCAGTATGTCTGAACCACAAAA-3’

PTR: 5’-TTGAATTTCGGTTGAGAACTCCTGGTTAATAGAAAAAT-3’

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a, the purified merT PCR fragment and the purified rpsT PCR fragment were mixed in a 1:1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pT. E. coli BL21 cells harboring the plasmid pT were named BL21-T.

For constructing the plasmid pP, primers PPF and PPR were used to amplify the gene merP using plasmid p5 as a template. The PCR fragment was purified.

PPF: 5’-AACTTTAAGAAGGAGTCATCATGAAGAAACTGTTT-3’

PPR: 5’-TTGAATTTCGGTTGATATTTGTTGCCTTACTTCTT-3’

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a, the purified merP PCR fragment and the purified rpsT PCR fragment were mixed in a 1:1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pP. E. coli BL21 cells harboring the plasmid pP were named BL21-P.

For constructing the plasmid pB1, primers PB1F and PB1R were used to amplify the gene merB1 using p5 as a template. The PCR fragment was purified.

PB1F: 5’-AACTTTAAGAAGGAGATGGACAAGACTATTTATTCCAAA-3’

PB1R: 5’-TTGAATTTCGGTTGAAGTACGCATTCATACTGGGCTTT-3’

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a, the purified merB1 PCR fragment and the purified rpsT PCR fragment were mixed in a 1:1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning

reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pB1. E. coli BL21 cells harboring the plasmid pB1 were named BL21-B1.

For constructing the plasmid pB2, primers PB2F and PB2R were used to amplify the gene merB2 using plasmid p5 as a template. The PCR fragment was purified.

PB2F: 5'-AACTTTAAGAAGGAGATGAAGCTCGCCCCATATATTTTA-3'

PB2R: 5'-TTGAATTTCGGTTGACCGATCACGGTGTCCTAGATGACAT-3'

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a, the purified merB2 PCR fragment and the purified rpsT PCR fragment were mixed in a 1:1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pB2. E. coli BL21 cells harboring the plasmid pB2 were named BL21-B2.

For constructing the plasmid pTP, primers PTPF and PTPR were used to amplify the gene cluster merT-merP using p5 as a template. The PCR fragment was purified.

PTPF: 5'-AACTTTAAGAAGGAGCCAGTATGTCTGAACCACAAAA-3'

PTPR: 5'-TTGAATTTCGGTTGATATTTGTTGCCTTACTTCTT-3'

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a, the purified merT-merP PCR fragment and the purified rpsT PCR fragment were mixed in a 1:1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pTP. E. coli BL21 cells harboring the plasmid pTP were named BL21-TP.

For constructing the plasmid pB1B2, primers PB1B2F and PB1B2R were used to amplify the gene merB1-merB2 using plasmid p5 as a template. The PCR fragment was purified.

PB1B2F: 5'-AACTTTAAGAAGGAGATGGACAAGACTATTTATTCCAAA-3'

PB1B2R: 5'-TTGAATTTCGGTTGACCGATCACGGTGTCCTAGATGACAT-3'

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a, the purified merB1-merB2 PCR fragment and the purified rpsT PCR fragment were mixed in a 1:1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pB1B2. E. coli BL21 cells harboring the plasmid pB1B2 were named BL21-B1B2.

For constructing the strain BL21-1, primers P211F and P211R were used to amplify the gene ppk-rpsT using p5 as a template. The PCR fragment was purified.

P211F:
5’-AATGGGTCGCGGATCCTTAACTTTAAGAAGGAGATGGGTCAGGAAAAGTTATATAT-3’

P211R:
5’-GTGCGGCCGCAAGCTTGAATTCAAGAAAATATCGAAGAAAAATAAATAAAGAGATT-3’

The plasmid pET28a was digested with Bam HI and Hind III. The digested plasmid pET28a and the purified ppk-rpsT PCR fragment were mixed in a 1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named p21-1. E. coli BL21 cells harboring the plasmid p21-1 were named BL21-1.

For constructing the plasmid pK, primers PKF and PKR were used to amplify the gene cluster ppk-rpsT using plasmid p5 as a template. The PCR fragment was purified.

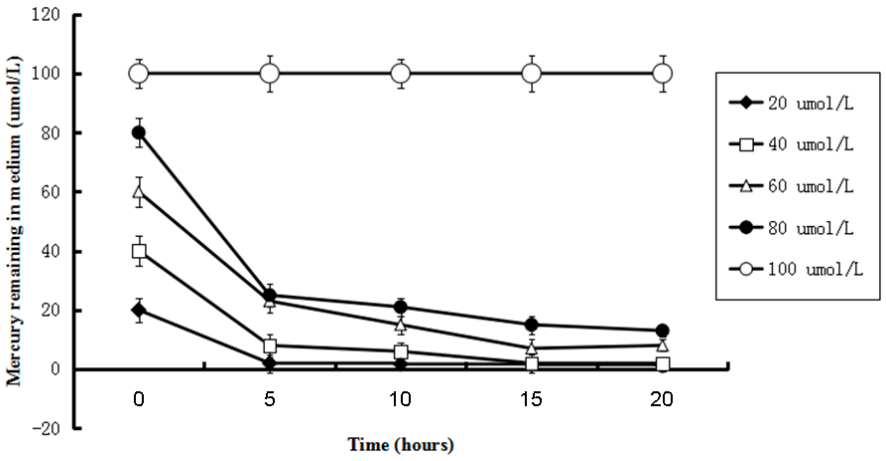
PKF: 5’-AACTTTAAGAAGGAGATGGGTCAGGAAAAGTTATATA-3’

PKR: 5’-GTGCGGCCGCAAGCTTGAATTCAAGAAAATATCGAA-3’

The plasmid pET28a was digested with Bam HI and Hind III. The synthesized sequence linker-P16S-g10, the digested plasmid pET28a and the purified ppk-rpsT PCR fragment were mixed in a 1:1:1 ratio for the in-fusion cloning reaction. In-fusion cloning reactions were performed according to the In-Fusion HD Cloning Kit User Manual (Takara, Dalian, China). Competent E. coli BL21 cells were transformed with the reaction mixture. Three clones on the LB plates containing 50 mg/L kanamycin were sent to the Sinogenomax Company (Beijing, China) for sequencing. Those plasmids with the predicted sequence were named pK. E. coli BL21 cells harboring the plasmid pTPK were named BL21-2.

S3: Hg remaining in medium after BL21-7 was cultured in LB liquid medium containing mercurial derivatives.

A shows BL21-7 cultured in LB liquid media containing HgCl2. B represents BL21-7 cultured in the presence of PMC. C shows BL21-7 cultured in the presence of MMC. MRM was measured when bacteria were cultured for 0 h, 5 h, 10 h, 15 h and 20 h, respectively.



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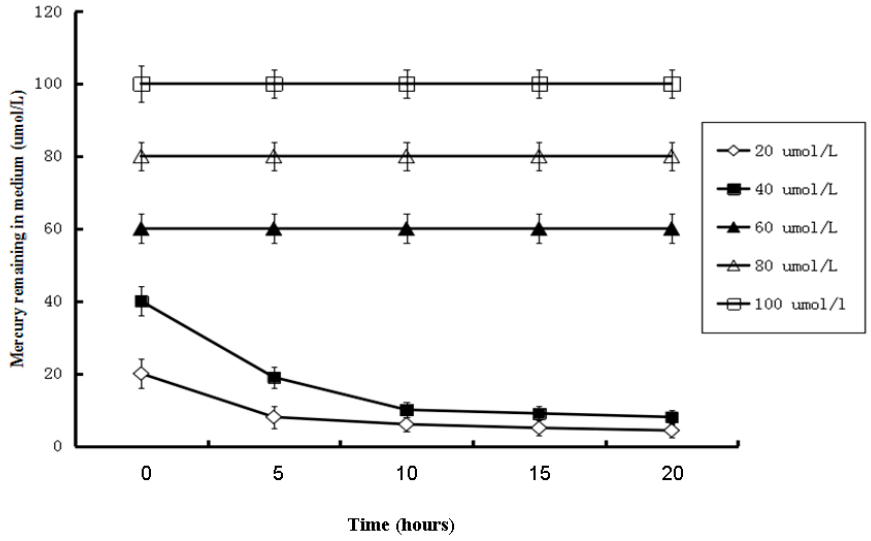

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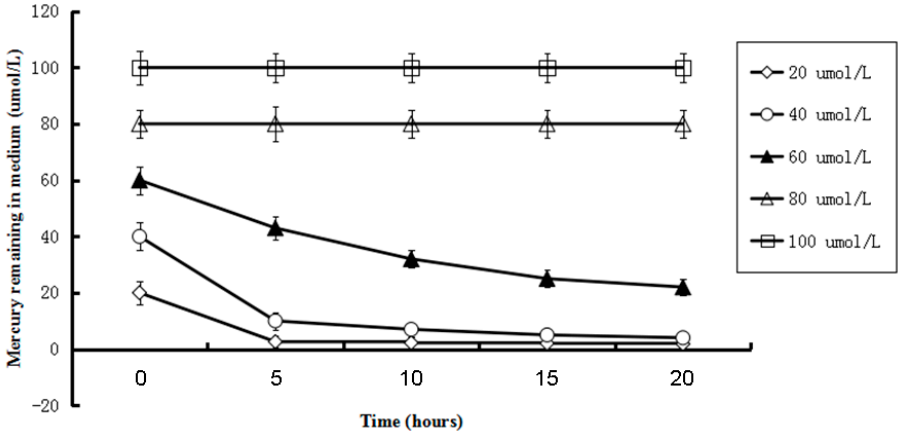

c