Supporting material

Disruption of Putrescine Biosynthesis in *Shewanella oneidensis* Enhances Biofilm Cohesiveness and Performance in Cr(VI) Immobilization

Yuanzhao Ding,[1,2] Ni Peng,[3] Yonghua Du,[4] Lianghui Ji,[3] Bin Cao[1,5,*]

[1]Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore

[2]Interdisciplinary Graduate School, Nanyang Technological University, Singapore

[3]Temasek Life Sciences Laboratory, Singapore

[4]Institute of Chemical and Engineering Sciences, A*STAR, Singapore

[5]School of Civil and Environmental Engineering, Nanyang Technological University, Singapore

***Corresponding author:** Bin Cao, Singapore Centre on Environmental Life Sciences Engineering, and School of Civil and Environmental Engineering, Nanyang Technological University, Singapore 637551. Fax: +65 6316 7349; Tel: +65 6592 7895.

E-mail: bincao@ntu.edu.sg

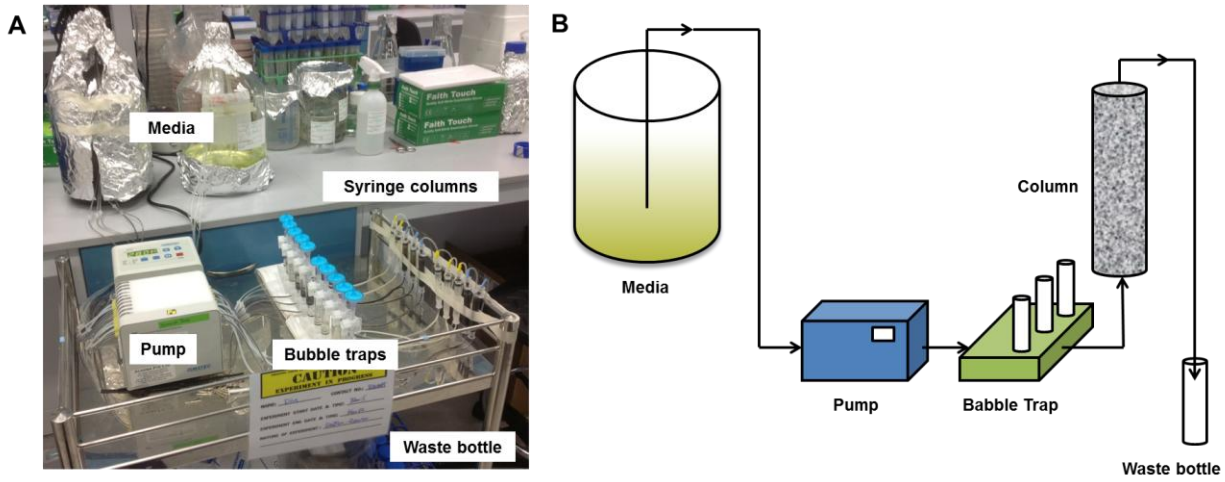
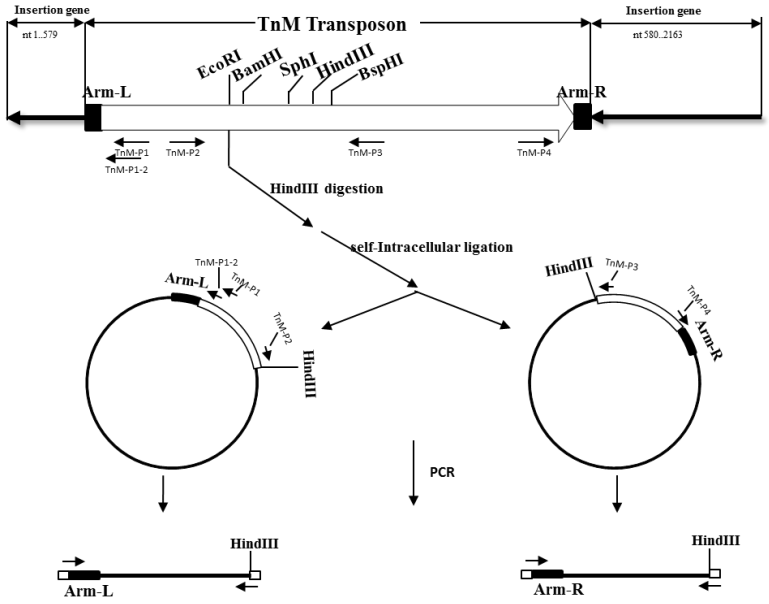


Fig. S1. (A) Experimental setup and (B) a schematic illustration of the submerged biofilm reactors used in this study.



33

34 **Fig. S2.** Illustration of the inverse PCR method used in this study. Inverse PCR
35 products were purified with Qiagen QIAquick Gel Extraction Kit and subsequently used
36 as templates for sequencing analysis. DNA sequencing was carried out using the same
37 primers adapted in inverse PCR.

38

39

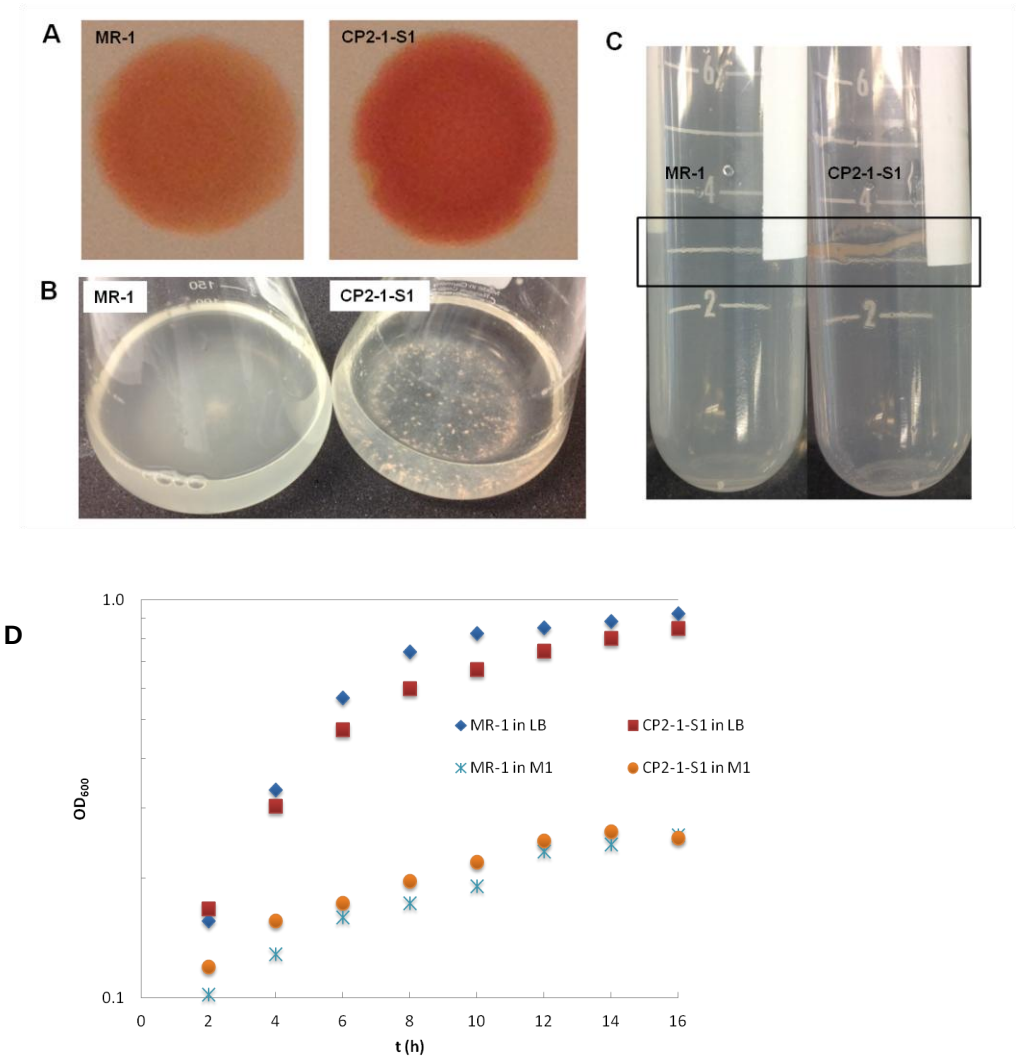


Fig. S3. The hyper-adherent phenotype of the mutant strain CP2-1-S1 (compared to *S. oneidensis* MR-1 WT): (A) Colonies formed on MM1 agar containing Congo red dye after 5-day incubation at 30°C; (B) 16-h planktonic cultures in MM1 medium; (C) Cell adhesion onto the wall of culture tubes in 16-h planktonic cultures in MM1 medium; (D) Representative growth profiles of the WT and CP2-1-S1 in LB and MM1 medium (growth assay in a 96-well plate with shaking).

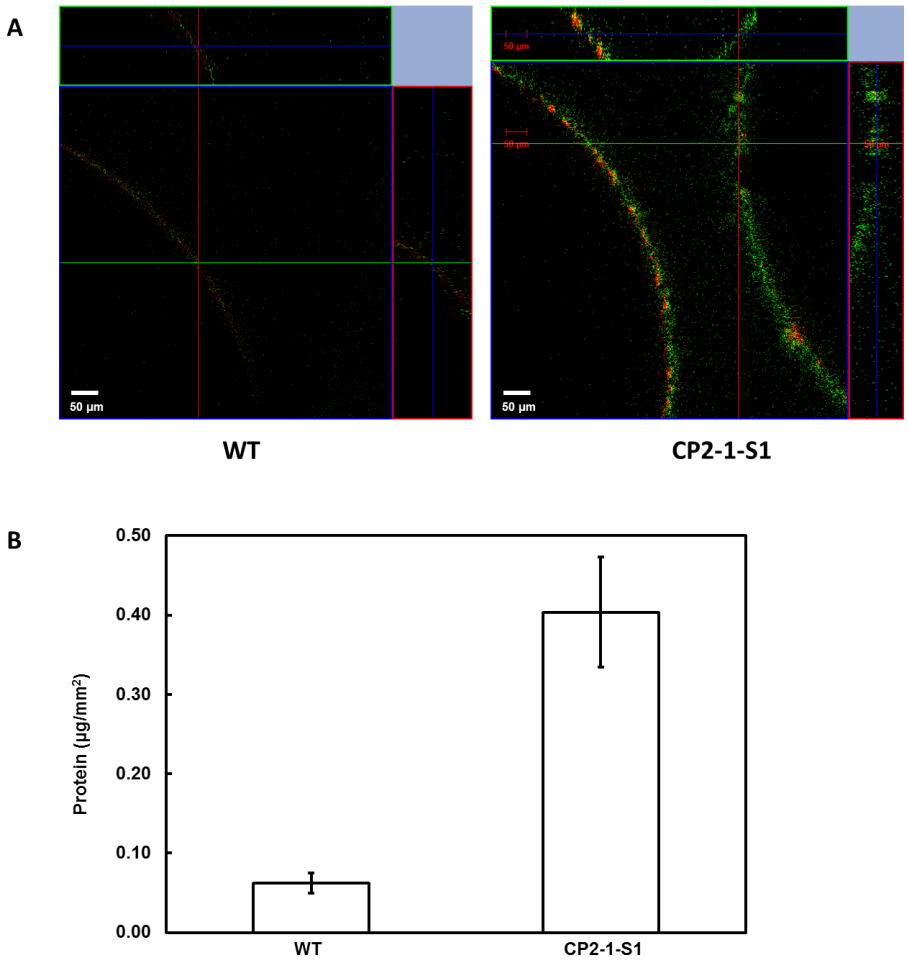


Fig. S4. Comparative analysis of the biofilms formed by CP2-1-S1 and the WT on glass beads in submerged biofilm reactors: (A) representative CLSM images. Live cells were stained green and dead cells were stained red and yellow with the LIVE/DEAD stain. (B) total amount of proteins per mm^2. Data are presented as means ± standard deviations (n = 3).

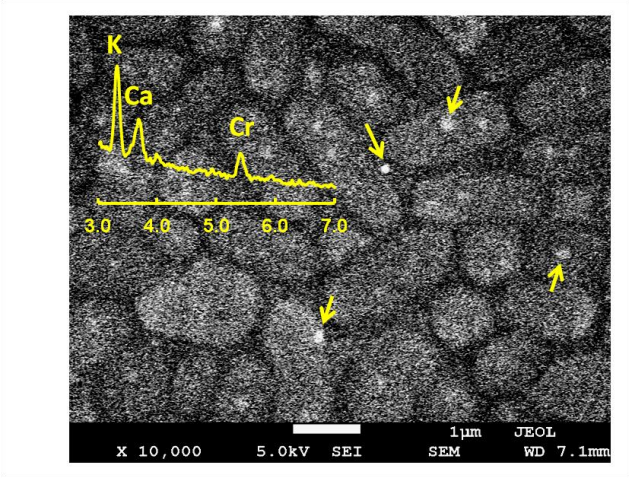


Fig. S5. A representative SEM-EDX spectrum showing Cr precipitates (indicated by arrows) in *S. oneidensis* biofilms after 56-h exposure to Cr(VI).

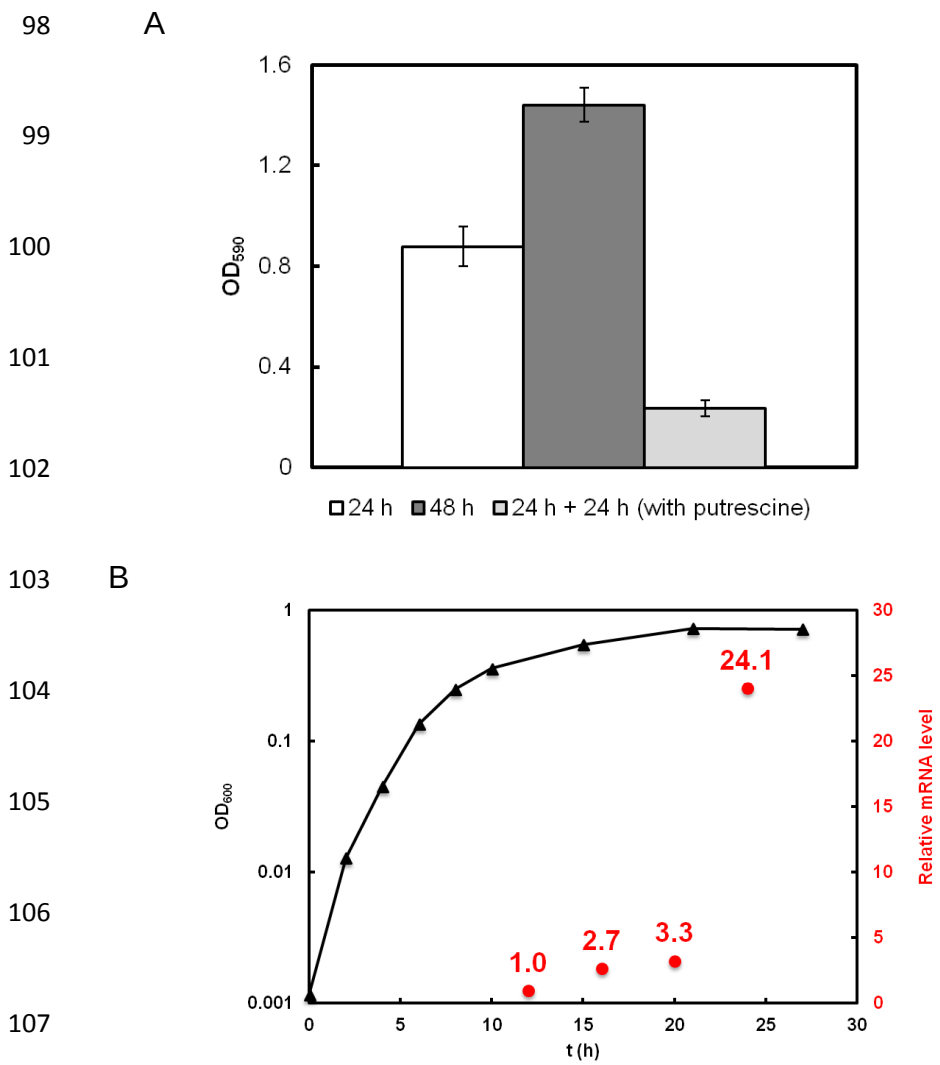


Fig. S6. (A) Dissolution of CP2-1-S1 biofilms by putrescine: CP2-1-S1 was growing for 24 h in a 96-well plate biofilm assay; then putrescine was added (final concentration 1 mM) and the cultures were incubated for another 24 h. (B) Expression of *speF* in *S. oneidensis* MR-1 growing in LB medium (planktonic cultures) revealed by qPCR analysis.

Table S1. Primers used for inverse PCR amplification.

Primer	Sequence
TnM-P1	5'-CGACATCATAACGGTTCTGGCA-3'
TnM-P1-2	5'-TGTGTGGAATTGTGAGCGGATA-3'
TnM-P2	5'-CACCGTGCAGTCGATGATAAGC-3'
TnM-P3	5'-CGCGCAGATCAGTTGGAAGAAT-3'
TnM-P4	5'-CCGCCACCTAACAATTCGTTCAA-3'