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Cadmium Adsorption *E. coli* with Surface Displayed CadR

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CadR is a metal-binding protein first isolated from *rhizobacterium pseudomonas putida* and specifically recognizes Cd^{2+}. *Escherichia coli* cells surface engineered with CadR show a high Cd^{2+} adsorption capacity of 19.5 μmol g^{-1} cells. The surface engineered *E. coli* cells also show higher tolerance towards cadmium contamination for up to 100 mM and could be potentially utilized as a bio-remediation treatment of cadmium contamination.

Cadmium is a non-essential toxic heavy metal and ranks as the sixth most toxic substances for significant human health hazard by U.S. Poison and Disease registry.[1] Cadmium intoxication symptoms range from respiratory tract, kidney dysfunction to notorious "*Itai-Itai*" disease which is caused by osteoporosis.[2-5] Evidences also show that some Cd^{2+}-containing compounds are carcinogenic.[6-10] Cadmium pollution mainly comes from mine drainage, electro-plant and battery industry waste.[11-12] Comparing with other metal ions, Cd^{2+} is easier to be absorbed by plants and further enriched in animals through food chain, its pollution is of priority in the World Health Organization's study of food contamination.[13-16] Japan and China are countries of most reported environmental cadmium exposures, partly due to their habit of eating rice.[17-23] Conventional cleanups of Cd^{2+} usually include chemical precipitation which are often ineffective for diluted waste water, and the precipitation itself could be of secondary polluting source.[24-26]

Bioremediation is a waste managing technology involving using natural- or genetically-modified microorganisms and/or plants for environmental cleanup and has been applied in ameliorating cadmium pollution.[27-28] The efforts vary from screening of marine microalgae to cultivation of plant growth-promoting Rhizobacteria.[29-33] However, heavy metal ions like Cd^{2+} are not readily absorbed or captured by natural organisms[34-39], we want to develop a genetically engineered microorganism with fast grow rates, high Cd^{2+} tolerance and surface area-volume ratio to obtain high cadmium removal efficiency.

CadR is a MerR family Cd^{2+}-binding protein first isolated from the *rhizobacterium pseudomonas putida 06909*, which regulates the cellular Cd^{2+} concentration by regulating the expression level of CadA, a Cd^{2+} efflux ATPase.[40-42] CadR contains 147 amino acids and three domains: the DNA binding domain, the metal binding domain and the coupling domain. Three cysteine residues (Cys 77, 112, 119) and its Histidine rich C-terminus are predicted as possible Cd^{2+} binding sites. The sensitive and specific recognition of Cd^{2+} by CadR has been developed as Cd^{2+} sensors.[43-45]

Herein, we want to report the construction of a surface-fused truncated CadR-OmpA on *E. coli* (Fig. 1).[46-48] These genetically-engineered *E. coli* cells show high Cd^{2+} tolerance and high adsorption capacity of Cd^{2+}. This system may be further developed into potential bio-remediation of Cd^{2+} contaminations.

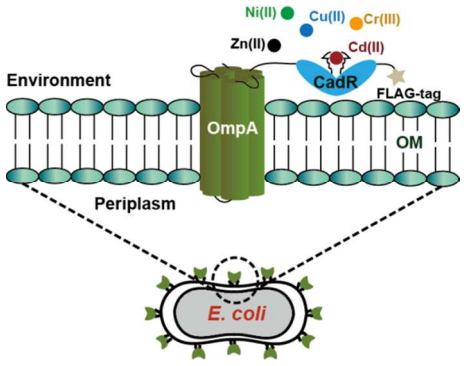

Fig. 1. Schematic representation of the CadR displayed on *E. coli* cell surface *via* the membrane protein OmpA. The yellow-green columns represent the membrane-spanning domain of OmpA and the blue cartoon is CadR displayed on the outer membrane of the cell.

To optimize the best CadR fraction for surface-display, we first tested Cd^{2+} binding affinity with full-length and truncated CadRs. The truncations were positioned to avoid perturbing of metal binding domain and were schematic represented in Fig. 2a. TC21 truncated

the random coil region of 21 amino acids at C-terminus and TC68 truncated DNA-binding domain of 68 amino acids at the N-terminus. Electrophoretic mobility shift assays showed that both full-length CadR and TC21 bind its promoter *pcadR* (Fig 2b) and the DNA-protein complex dissociated upon addition of Cd^{2+} (Fig 2c). Metal ions such as Ni^{2+}, Cu^{2+} and Cr^{3+} were ineffective on dissociating CadR from CadR-DNA complex while Zn^{2+} was less effective than Cd^{2+}(Fig 2d). TC68 lost the binding ability of *pcadR* due to the truncation of DNA binding domain. Isothermal titration microcalorimetry (ITC) was used to measure the changing of observed enthalpy (ΔH_{obs}) by titrating Cd^{2+} (0.25 mM) into the TC21 solution (0.05 mM) (Fig 2e). When the molar ratio of TC21 to Cd^{2+} reached ~1:2, the ΔH_{obs} values reached a platform, which agreed with previous report of CadR dimer formation upon Cd^{2+} binding.[40, 42] The association constant of TC21 with Cd^{2+} is about 1.47×10^7 M^{-1}. ITC measurements of CadR gave similar results as TC21 (Fig S2), while TC68 showed weaker binding which may cause by miss-folding or instability of the protein under the ITC conditions (Fig S3). The Tris-buffer and His-tag casts negligible influences on the ITC results through the four control titrations of Cd^{2+} to buffer, His-tag labeled proteins and GST-labeled protein (Fig S4, S5, S6). When three key cysteine residues (C77, C112, C119) at the C-terminus were mutated to serine respectively, ITC measurements showed dramatically decreased Cd^{2+} binding, which confirmed the importance of these cysteine residues (Fig S8, 9, 10).The cysteine residues could tolerate limited oxidative environment since all our experiments were carried out in the air, no reducing agents were added for dialysis and ITC experiments.

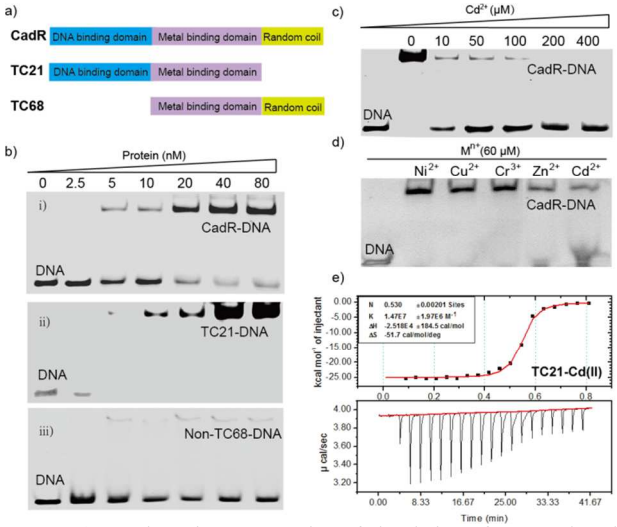

Fig. 2. a) A schematic representation of the design of truncated CadRs. b) Electrophoretic mobility shift assays of the binding of i) CadR, ii) TC21 and iii) TC68 to *pcadR*. c) The concentration dependent dissociation of CadR-*pcadR* complex by Cd^{2+}. d) Electrophoretic mobility shift assays of other metal ions with CadR-*pcadR*. e) ITC titration for TC21 with Cd^{2+}.

Following protocols reported by Zhao *et. al*,[48] we fused CadR, TC21 and TC68 with a C-terminus Flag-tag to the C terminus of OmpA(N1-159) respectively (Fig 1). After inducing with arabinose, the recombinant fusion proteins' expression in the membrane fraction was confirmed by SDS-PAGE with correct molecule weights (Fig 3a). Also, immunoblotting analysis verified the expression of TC68-OmpA expression with anti-FLAG antibodies (Fig 3b). Then the immunofluorescence experiments further confirmed the successful expression of fusion proteins on the *E. coli* extracellular surfaces (Fig 3c).

The engineered *E. coli* cells were further tested for their cadmium adsorption ability. After 2 hours' induction with 0.05% arabinose in

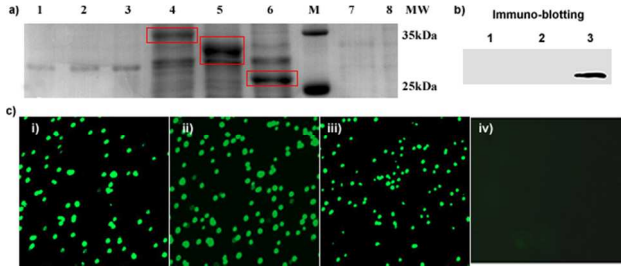

Fig. 3. Characterization of the surface-displayed proteins. a) SDS-PAGE analysis of surface-displayed protein. The red boxes indicate the three proteins, OmpA-CadR (lane 4, 34.8 kDa), OmpA-TC21 (lane 5, 33.6 kDa) and OmpA-TC68 (lane 6, 28.0 kDa). Lane 1-3 show the membrane fraction from the uninduced bacteria, OmpA-CadR (lane 1), OmpA-TC21 (lane 2) and OmpA-TC68 (lane 3). The supernatant protein fraction from induced (lane 7) or uninduced (lane 8) OmpA-TC68 bacteria are used as controls. b) The immunoblotting (anti-FLAG antibody) of OmpA-TC68-FLAG in membrane fraction of induced bacteria (lane 3), supernatant (lane 2) and the membrane fraction of the uninduced control (lane 1). c) Immunofluorescence labelling of *E. coli* cells using anti-FLAG antibody and FITC conjugated anti-mouse IgG antibody. i) Arabinose induced OmpA-CadR bacteria, ii) Induced OmpA-TC21 bacteria, iii) Induced OmpA-TC68 bacteria and iv) Uninduced OmpA-TC68 bacteria as control.

LB broth, the bacteria were treated with Cd^{2+} (60 μmol L^{-1}) for overnight. The harvested cells were washed three times with water and digested with microwave. Then the Cd^{2+} concentration was measured by ICP-MS. The Cd^{2+} adsorption efficiencies of the surface engineered bacteria were significantly higher than *E. coli* cells without CadR fusion (Fig 4a). The TC68-OmpA fusion construct showed the highest adsorption capacity of about 19.5 μmol g^{-1} cells. Comparing with CadR and TC21, TC68's small size may significantly improve the over-all display efficiency. Thanks to CadR's selectivity towards Cd^{2+}, the TC68 engineered *E. coli* cells showed 100-fold higher selectivity towards Cd^{2+} over Cu^{2+}, Ni^{2+} and Cr^{3+}, 10-fold higher selectivity over Zn^{2+} (Fig 4b).

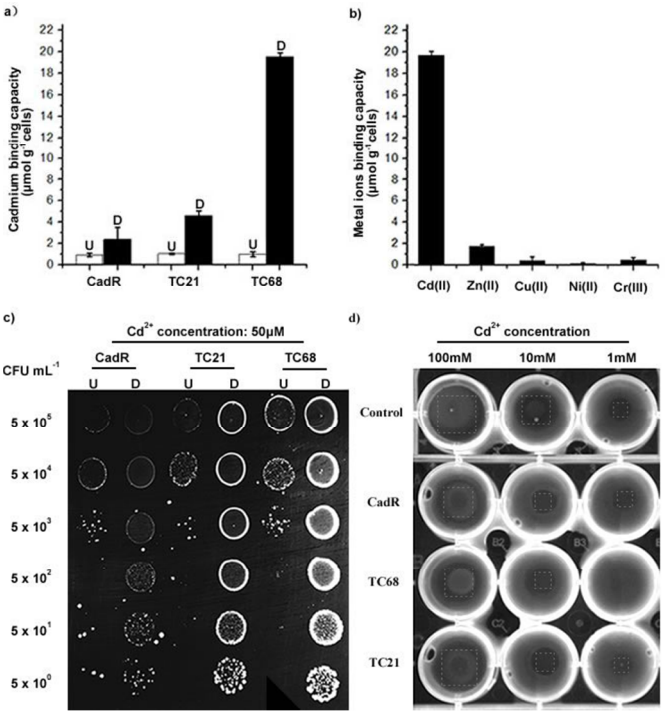

Fig. 4 a) Cd^{2+} adsorption capacity with the three engineered *E. coli* cells. The three surface displayed *E. coli* cells (OmpA-CadR, OmpA-TC21, OmpA-TC68) were labelled as D, the three controlled *E. coli* cells without OmpA engineering (CadR, TC21, TC68) were labelled as U. b) Adsorption of other metal ions with OmpA-TC68 cells in a same condition. c) Plate assays of Cd^{2+} tolerance with the *E. coli* cells same as in Fig. 4a. *E. coli* cells were spotted on solid medium containing 0.05% arabinose and 50 μM Cd^{2+}. The plate was incubated at 37 ℃ overnight before being read. d) Cd^{2+} tolerance of surface displayed *E. coli* cells (OmpA-CadR, OmpA-TC21, OmpA-TC68) under the treatment of different concentration of Cd^{2+}. Cd^{2+} solution was added on the solid medium containing induced *E. coli* cells and 0.05% arabinose. The plate was incubated at 37 ℃ overnight before being read. Dotted boxes indicate the suppression plaques, Smaller dotted boxes indicated more bacteria survival.

Notably, the surface engineered *E. coli* cells showed tremendously improved Cd^{2+} tolerance. Plate assays showed more than 1000 fold Cd^{2+} tolerance improvement for all three surface engineered *E. coli* cells (Figure 4c). The TC68 construct could even survive and grow without visible defect at the Cd^{2+} concentration at 1 mmol L^{-1} (Fig 4d)! This finding suggested that the surface displayed CadR motif could adsorb Cd^{2+} and protect the cells from cadmium intoxication, which is crucial for its potential applications in cadmium pollution remediation.

Conclusions

In summary, CadR, a Cd^{2+} selective metalloprotein was displayed on *E. coli* cell surface and the surface engineered *E. coli* cells showed high tolerance of Cd^{2+} intoxication and high Cd^{2+} adsorption capability. This method may have potential application as selective Cd^{2+} biosorbent and this concept could be applied to other selective metal binding motives and other organisms.

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Notes and references

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