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Bioremediation potential of cadmium by recombinant *Escherichia coli* surface expressing metallothionein MTT5 from *Tetrahymena thermophila*

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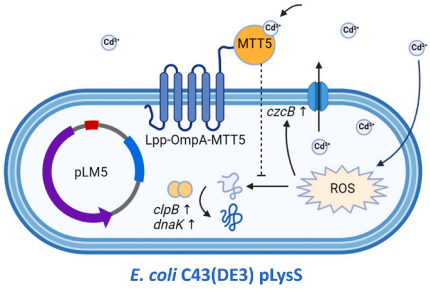
HIGHLIGHTS

- Invertebrates MTs expressed on *E. coli* surface for Cd adsorption was first set up.
- Our recombinant *E. coli* with surface MTT5 shows a higher adsorption than wild type.
- MTT5 could chelate with Cd^{2+}, and decrease the induction of *dnaK* and *clpB*.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium (Cd) is a common heavy metal contaminant in industrial wastewater that causes many diseases in humans. Metallothionein (MT), a cysteine-rich metal-binding protein, is well known in chelate-heavy metals. In this study, we expressed MTT5 of *Tetrahymena thermophila* fused with Lpp-OmpA in the outer membrane of *Escherichia coli* to determine its ability to accumulate and adsorb Cd. Our results revealed that our recombinant *E. coli* had a 4.9-fold greater Cd adsorption compared to wild *E. coli*. Adsorption isothermic analysis demonstrated that the adsorption behavior for Cd in our recombinant bacteria was better fitted into the Freundlich isotherm model than Langmuir isotherm model. Fourier-transform infrared spectroscopy indicated that phosphate and organic phosphate groups were involved in the interaction between Cd and the bacterial surface. Using quantitative reverse transcription polymerase chain reaction, we further showed that the expression of metal-resistance genes (*dnaK* and *clpB*) was downregulated due to surface MTT5 protected our recombinant bacteria from Cd^{2+} adsorption. Furthermore, we showed that our recombinant bacteria could adsorb Cd from the contaminated wastewater containing other metals and were suggested to be applied in the field study.

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1. Introduction

Heavy metal contamination is a primary global environmental concern (Afzal et al., 2017; Rajendran et al., 2022). The main sources of heavy metal contamination are anthropogenic activities: mining, electroplating, plastic stabilizers, manufacturing batteries, alloy, pigment, cement, fossil fuel combustion; sewage sludge incineration; and high-phosphate fertilizers (Khan et al., 2016). Release of these metals is a significant issue as they are a severe threat to the health and well-being of humans and animals (Bressa et al., 1988; Selvin et al., 2009). For example, cadmium (Cd) is a heavy metal mainly found in industrial wastewater (Smrithi and Usha, 2012). Cd could induce DNA double-strand breaks, leading to genome instability (He et al., 2014; Ahmed et al., 2021; Priyadarshanee and Das, 2021). In addition, it causes many diseases, including osteoporosis, anemia, eosinophilia, anosmia, apoptosis, diabetes mellitus, oncogenic activation, itai-itai disease, and chronic pulmonary problems (Khan et al., 2015).

Several chemical and physical approaches have been applied to eliminate Cd^{2+} from industrial wastewater. However, these approaches were costly and relatively ineffective (Ahmed et al., 2021; Priyadarshanee and Das, 2021). Adsorption is a prospective process for toxic heavy metals elimination because of its simplicity, cost-effectiveness, and eco-friendliness (Hassan et al., 2018; Han et al., 2022). Biosorption has been used to sequestrate heavy metals from contaminated sites with evenly diluted aqueous solutions through physicochemical processes (Volesky, 2007). Many studies using microbial biomass (bacteria, fungi, or algae) to remove heavy metal ions from contaminated sewages were described (Hassan et al., 2018; Ahmed et al., 2021; Priyadarshanee and Das, 2021). Biosorption can be classified as a metabolism-independent process. On the other hand, the removal of Cd^{2+} by bacteria has also been achieved through metabolism-dependent intracellular accumulation (Khan et al., 2016). Hyper-accumulation of Cd^{2+} in bacteria induces reactive oxygen species (ROS) and damages the respiratory chain (Gibbons et al., 2011; Zeng et al., 2012; Mansoor et al., 2021; Zulfiqar et al., 2022). Some bacteria are equipped with resistance mechanisms to counteract the detrimental effects of intracellular Cd^{2+} accumulation, including efflux transport, precipitation, and intracellular sequestration by metallothionein (MT), glutathione, and other thiol-containing compounds (Nies, 1995; Intorne et al., 2012; Maynaud et al., 2014).

MT, a cysteine-rich (20–30%) protein, can sequester of metal ions (Klaassen et al., 2009; Chaturvedi et al., 2012). The sequences of MTs from different species are somewhat different. The expression level of these MTs can be increased by heavy metals and other environmental stressors (Zhou et al., 2017). Compared with vertebrate MTs, multiple isoforms of MT exist in invertebrates (Amiard et al., 2006). Mice harbor 4 MT genes, and humans possess at least 16 MT genes clustered on Chromosome 16 (Palmiter, 1998). Meanwhile, 41 MTs have been reported from among 13 *Tetrahymena* spp. (de Francisco et al., 2016). However, only a few studies have evaluated the potential of invertebrate MTs in bioremediation (Pérez-Rafael et al., 2012; Turchi et al., 2012; Chaturvedi and Archana, 2014; He et al., 2014). The expression of MTT5 from *Tetrahymena thermophila* could be used as a candidate for Cd bioremediation (Díaz et al., 2007; Zhou et al., 2017, 2018). Several studies have shown that recombinant *E. coli* enhanced the bioaccumulation ability of Cd^{2+} through MT expression in the cytosol (Kim et al., 2005; Deng et al., 2007). Engineered bacteria expressing MTs have promising bioremediation applicability (Ma et al., 2019). However, the expression efficiency of functional MT proteins in cytosol is limited (Ma et al., 2019), due that the expression of MTs in bacterial cytosol was easily disrupted by the cysteine thiol groups of MTs oxidized, leading to MT proteolysis during expression in the host cells (Morris et al., 1999; Suleman and Shakoori, 2012; He et al., 2014).

We examined the potential of *E. coli* expressing *T. thermophila* MTT5 in the cytoplasmic and outer membrane compartments for Cd^{2+} accumulation and adsorption, respectively. MTT5 was expressed as a fusion protein with a lipoprotein signal sequence, the first nine amino acids of lipoprotein (Lpp), and the region between residue 46 and 159 amino acids of outer membrane protein A (OmpA) of *E. coli* (Francisco et al., 1993). Therefore, MTT5 could be expressed on the bacterial surface with the help of Lpp-OmpA. Compared to intracellular expression of MTT5, surface expression of MTT5 could show higher Cd adsorption due to timesaving for crossing the membrane and avoiding disturbance of the related redox pathways for Cd removal in the cytosol Tafakori et al. (2012) described.

Many reports were focused on the effects of MTs of vertebrate or prokaryotes on the chelation of heavy metals (Lavradas et al., 2014; Wang et al., 2014; Comes et al., 2019) However, these effects were seldom reported for MTs of invertebrate in chelating heavy metals. Although MTT5 of invertebrate *Tetrahymena hemophilia* has been suggested to chelate Cd^{2+}, the application of MTT5 on Cd^{2+} removal remains unexplored. The objectives of this study were to a) characterize the recombinant *E. coli* strain expressing MTT5 and its resistance to and biosorption of Cd^{2+}, b) ascertain the resistance mechanism to Cd^{2+} ions by analyzing the alteration in Cd^{2+} resistance gene expression levels.

2. Materials and methods

2.1. Strains, medium, and culture conditions

All strains and plasmids employed in this study are listed in Supplementary Table S1. *E. coli* strain C43(DE3) with plasmid pLysS (Lucigen Inc., USA) and all its recombinants were cultured with pLO, pLM5 and pET-MTT5 (Supplementary Table S1) at 37 °C in Luria-Bertani (LB) medium with 200 rpm shaking. An antibiotic (34 μg/mL of chloramphenicol and 100 μg/mL of ampicillin) was added into LB medium during cultivation and protein expression.

2.2. Construction and expression of chimeras

A fragment containing Lpp-OmpA was amplified from *E. coli* chromosome using overlap extension polymerase chain reaction (PCR) (Yang et al., 2008) with primers Lpp up F, Lpp down R, OmpA up F and OmpA down R (Supplementary Table S2). 435 bp of Lpp-OmpA PCR product was digested with *NdeI* and *BamHI* and ligated along a pET21b vector, and the resulting plasmid was pLO. *T. thermophila* metallothionein (*mtt5*) gene (Zhou et al., 2017) was chemically synthesized by MDBio, Taipei, Taiwan. The synthetic *mtt5* gene was inserted into the *BamHI/EcoRI* of pLO and pET21b; the resulting plasmids were pMTT5 and pET-MTT5, respectively. Plasmid pLO, pET-MTT5 and pMTT5 were transformed (Chung et al., 1989) into TSS-competent *E. coli* strain C43 (DE3) for protein expression.

The Lpp-OmpA, MTT5 or Lpp-OmpA-MTT5 fusion proteins were expressed in *E. coli* strain C43 (DE3). Overnight cultures of the transformed strains were diluted at a 1:100 ratio into LB medium. After OD_{600} value of incubation reached 0.6 at 37 °C and 200 rpm shaking, the cultures were induced to express membrane protein by adding mmole/L of isopropylbeta-D-thiogalactopyranoside (IPTG) and incubated at 30 °C and 200 rpm shaking. The cells were harvested by centrifugation after 4 h induction for membrane protein fractionation.

2.3. Membrane protein fractionation

To extract the chimeric proteins from the outer membrane of *E. coli* C43 (DE3), cells were fractionated following ReadyPrep™ Protein Extraction Kit (Membrane II, Bio-Rad, California, USA). After induction and harvesting, the cells that expressed membrane protein were resuspended in 50- mmole/L Tris–HCl buffer (pH 7.3). For the cell lysates, the cells were lysed on ice with a Q125 sonicator (Qsonica, Connecticut, USA). The crude extracts were centrifuged for 10 min at 3000 g to remove cell debris. Supernatants were added into 15 mL of membrane protein concentration reagent (Bio-Rad, California, USA) and then

incubated on ice for 1 h. After ultracentrifugation (100,000 g at 4 °C for 1 h), the cellular membrane protein pellet was resuspended into 2-D rehydration/sample buffer (7 mol/L urea, 2 mol/L thiourea, 1%(m/v) ASB-14, 40 mmol/L Tris, 0.001%(m/v) Bromophenol Blue) for subsequent experimentation.

2.4. Western blotting for membrane protein

In the Western blot analysis, the purified membrane protein concentrations were quantified using an RC DC™ Protein Assay (Bio-Rad, California, USA). The membrane protein samples (30 μg) were electrophorized by 12%(m/v) SDS poly-acrylamide-gel-electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes with a pore size of 0.45 μm (Millipore, Burlington, USA). The membranes were blocked with 2%(m/v) bovine serum albumin (BSA; Sigma-Aldrich, California, USA) in phosphate-buffered saline and 0.05%(m/v) Tween 20 (PBST) for 1 h at 4 °C. 6x His-tag monoclonal antibody (Invitrogen, Carlsbad, CA, USA) was diluted in 2%(m/v) BSA/PBST at a 1:5000 ratio and incubated with membranes at 4 °C for 1 h. After three washes with PBST to remove antibodies, the membranes were rinsed with SuperSignal West Pico PLUS agent (Thermo Fisher Scientific, California, USA) for bound antibody visualization.

2.5. Metal adsorption capacity by surface-engineered cells

To determine the potential of MT on the cellular surface for Cd absorption, we performed a metal adsorption assay (Sedlakova-Kadukova et al., 2019). *E. coli* C43 (DE3) cells with pLO, pLM5 and pET-MTT5, respectively, were cultivated in LB medium and induced with IPTG at an OD_{600} of 0.6. After 4 h, the cells were collected by centrifugation (4000 g) and pellets were washed twice with 10 mL PBS. Subsequently, the cells were suspended in 50 mL of LB medium with different concentrations (0, 5, 10, 20, 40, and 80 mg/L) of $CdCl_2$ and incubated at 30 °C with 200 rpm for 3 h. The pellets were harvested with centrifugation (4000 g) and dried at 60 °C in an oven. The pellets and supernatant were digested by the mixtures of 5 mL of concentrated H_2SO_4 and 2 mL of concentrated HNO_3, and then were analyzed for the indicated concentrations of Cd^{2+} ions with an inductively coupled plasma-optical emission spectrometry (Optima 2100DV, PerkinElmer, Ohio, USA). All experiments were performed in triplicate.

2.6. Fourier-transform infrared spectroscopy (FT-IR)

To understand whether expressing membrane proteins affect the binding properties of bacterial surfaces, we treated 50 mL of *E. coli* C43 (DE3) pLM5 with 20 mg/L $CdCl_2$ for 3 h. Treated culture was centrifuged at 4000 g for 10 min and supernatant was removed. Cell pellets were dried at room temperature to make powder. Finally, the bacterial powder was directly analyzed using an Agilent Technologies Cary 630 FT-IR spectrometer (Agilent, California, USA).

2.7. Quantitative reverse transcriptase polymerase chain reaction (RT-qPCR)

E. coli C43 (DE3) with pLO or pET-MTT5 was treated with 0 and 20 mg/L $CdCl_2$ for 3 h, and cell pellets were collected by centrifuge (12,000 g for 1 min). Total RNA of cell pellets was purified with RNeasy Protect Bacteria Mini Kit (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was reverse transcribed from RNA with iScript™ Reverse Transcription Supermix (Bio-Rad, California, USA). We quantified the expression levels of *mutS*, *ef-tu*, *ftsZ*, *dnaK clpB*, *czcB* and *cca* genes using qPCR, while *cca* (tRNA nucleotidyltransferase) was employed as an internal control. All primers used for qPCR are listed in Supplementary Table S3 qPCR amplifications were performed with BioRad CFX Connect Quantitative real-time PCR (qPCR) system (BioRad, California, USA) and iQ SYBR® Green Super Mix Kit (BioRad, California, USA).

2.8. Statistical analysis

One-way ANOVA followed by a Tukey's test was performed using the *base* R package.

3. Results

3.1. Construction and expression of the MTT5 and Lpp-OmpA MTT5 in recombinant strains

We achieved MTT5 expression on the surface of *E. coli* surface using Lpp-OmpA signal peptide. The regulation of the T7 promoter using IPTG, MTT5 expression was induced (Supplementary Fig. S1). Construction of the recombinant plasmids was confirmed by Western blots using anti-His tag HRP antibody for membrane detection (Fig. 1). Without Lpp-OmpA signal peptides attached to MTT5, we detected MTT5 expression in the cytosol (data not shown). Taken together, our results clearly showed that chimeric Lpp-OmpA protein was expressed and localized to *E. coli* surface, while MTT5 protein *per se* was localized to the outer membrane.

3.2. Cd binding by recombinant E. coli strains expressing MTT5

For adsorption studies, metal concentrations at or below the minimal inhibition concentrations (MICs) are typically employed (Misra et al., 2021). Therefore, we used nontoxic concentrations of 20 mg/L for Cd^{2+} for the subsequent experiments based on the sensitivity of our recombinant bacteria to Cd^{2+} (Supplementary Fig. S2). We exanimated the adsorption of Cd by the recombinant stains to evaluate the effects of the MTT5 expression on cell surface using atomic adsorption analysis (Fig. 2). The adsorptive capacity (q_e) of C43(DE3) (host strain), pLO (empty vector), pET-MTT5 (MTT5 in cytosol), and pLM5 (MTT5 in outer membrane) were 0.0857, 0.2895, 0.3105, and 1.53 mg/g dry weight, respectively. The adsorption of pLM5 was 4.9-fold greater than that of

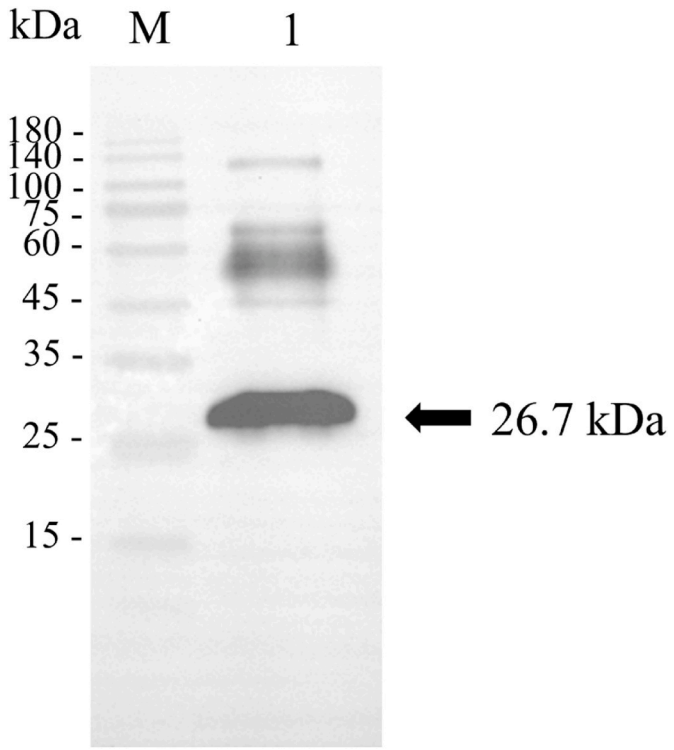

Fig. 1. Expression of LO-MTT5 in membrane fractions. Lane M: protein marker (kDa). Lane 1 represents membrane fraction of C43(DE3) pLM5. Desired fusion proteins marked with arrows.

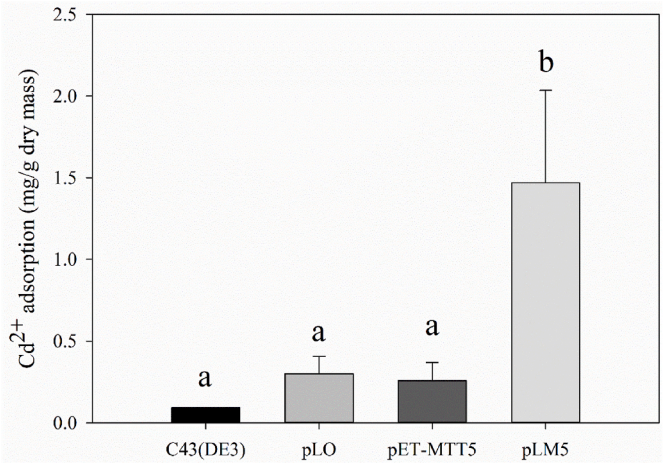

Fig. 2. Cd^{2+} adsorption ability of C43(DE3) pLysS carrying pLO (control), pET-MTT5 (cytoplasm-displayed) and pLM5 (outer membrane-displayed). Adsorption assay performed in LB medium containing 20 mg/L $CdCl_2$ at 30 °C for 3 h. All data expressed as mean ± SD (n = 3). ANOVA performed using R. Different lower-case letters within a group indicate significant differences (Tukey's test, $p < 0.05$).

pET-MTT5. We studied the adsorption of Cd^{2+} by pLM5 by varying initial Cd concentrations ranging from 20 mg/L to 80 mg/L. The adsorption capacity of pLM5 showed a linear relationship with the increasing concentrations of Cd^{2+} (Fig. 3). Therefore, we confirmed that Cd^{2+} could be chelated on the surface of *E. coli* through surface displayed MTT5. However, the Cd removal rate was reduced to approximately 23% when the Cd concentration was increased to 20 mg/L.

3.3. Adsorption isotherms

Regression lines were obtained when our data was analyzed with applying Langmuir and Freundich isotherm equations (Fig. 4), suggesting that our data fit both isothermic models. The values of the regression coefficients (R^2) for Langmuir and Freundich isotherm equations were 0.87 and 0.96, respectively, indicating that our data were better correlated with Freundlich's than Langmuir's equation.

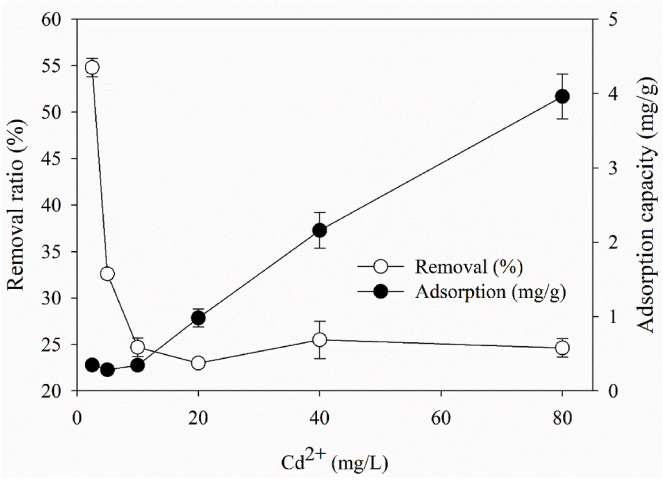

Fig. 3. Removal percentages and Cd^{2+} adsorption capacities of C43(DE3) pLM5 biosorbent at different Cd^{2+} concentrations (pH = 7, agitation speed: 200 rpm, process duration: 3 h, amount of adsorbent: 30 mg, temperature: 30 °C). All data expressed as mean ± SD (n = 3).

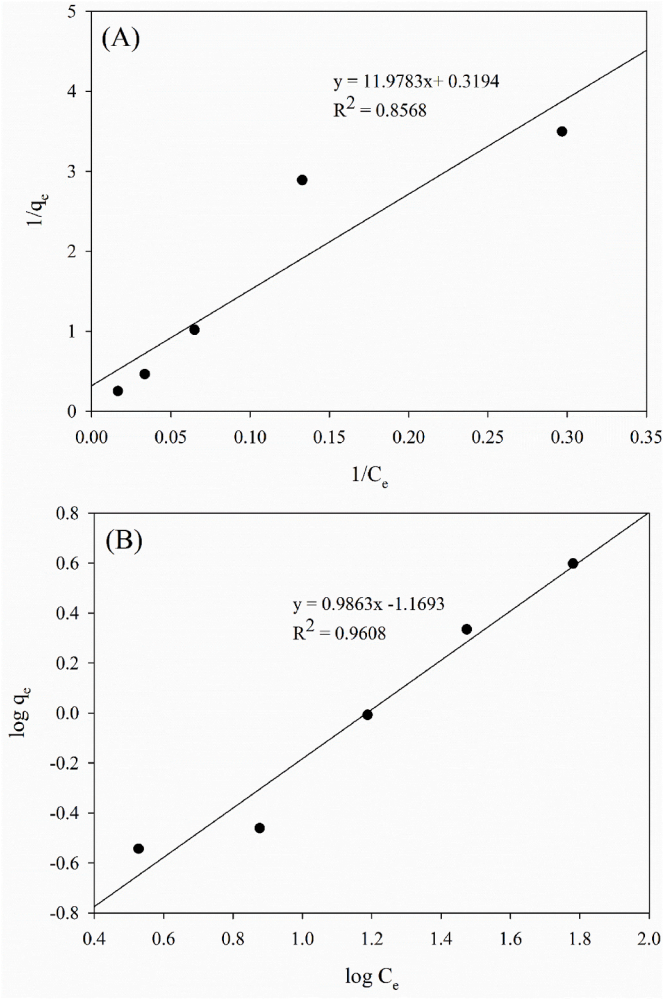

Fig. 4. Adsorption isotherms of C43(DE3) pLM5 biosorbent. (a)Langmuir isotherms and (b)Freundlich isotherms fitting plots.

3.4. FT-IR analysis

FT-IR analysis confirmed that carboxyl, amino, phosphate and organic phosphate moieties shifted. It was clear that the peaks attributed to carboxyl, amino, phosphate and organic phosphate moieties appeared at 1634, 1534.1230 and 1077 cm^{-1} without Cd^{2+} binding. The corresponding peaks moved to 1627, 1526, 1226 and 1058 cm^{-1}, respectively, after Cd^{2+} binding on the cell membrane (Fig. 5).

3.5. Expression of Cd resistance genes

The gene expression of *mutS, ef-tu, ftsZ, dnaK, clpB* and *czcB* were determined by their values normalized to *cca*, a housekeeping gene, using quantitative real-time PCR (Fig. 6). Compared to those genes in pLO (without Cd treatment), the expression of *dnaK*, *clpB*, and *czcB* significantly increased in the pLO with Cd treatment, while *czcB* was also expressed profoundly in pLM5 with Cd treatment.

3.6. Ability of Cd adsorption in wastewater

The ability of Cd adsorption with our recombinant bacteria in wastewater for 24 and 48 h, respectively, was determined. Cd wastewater was collected from electroplating factory and Supplementary Table S4 presented its characteristics. The wastewater contained 20 ppm of Cd, 4.23 ppm of nickel, 1.2 ppm of copper, and 126.6 ppm of chromium. After the treatment of 24 and 48 h, the adsorption of C43(DE3),

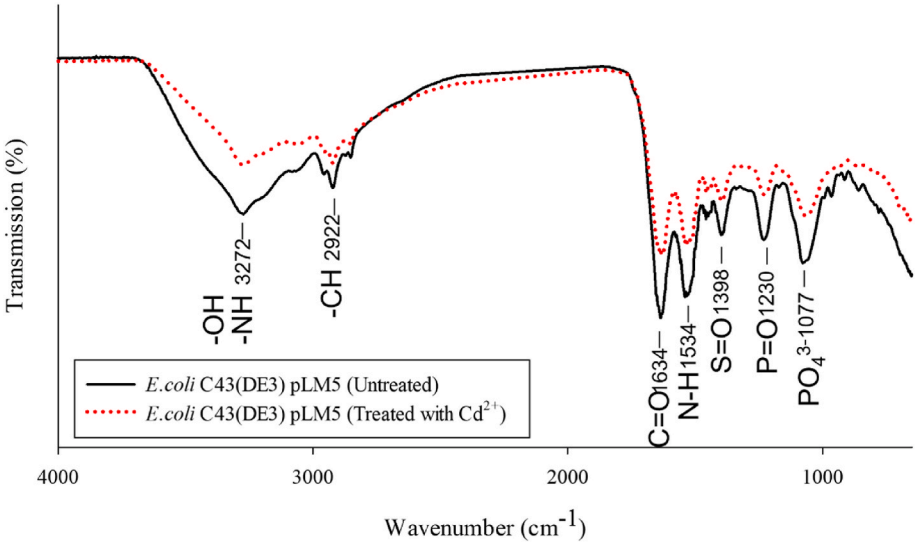

Fig. 5. FT-IR spectrum of C43(DE3) pLM5 biosorbents, untreated and treated with Cd^{2+}.

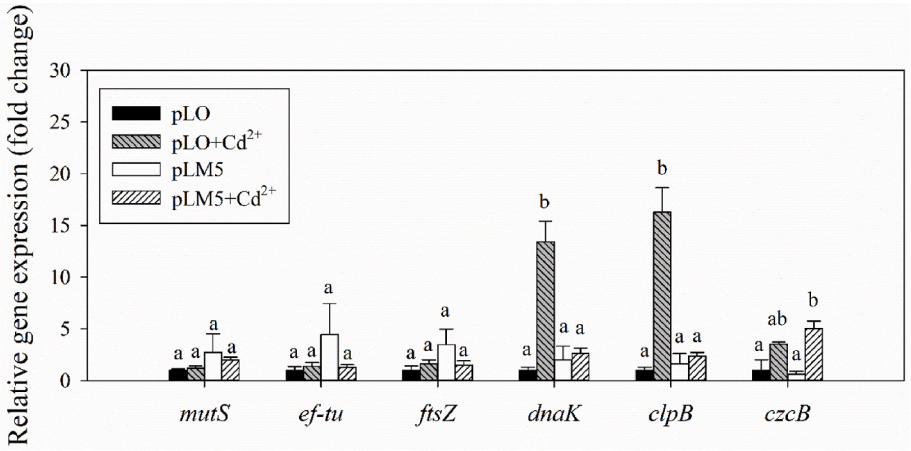

Fig. 6. Relative expression of *mutS, ef-tu, ftsZ, dnaK, clpB* and *czcB* determined by quantitative PCR. Gene expression levels for each gene compared to *cca* (tRNA nucleotidyltransferase). All data expressed as mean ± SD (n = 3). ANOVA performed using R. Different lower-case letters within a group indicate significant differences (Tukey's test, $p < 0.05$).

pLO, and pLM5 were 10.85, 14.2, and 19.1 mg/g dry weight as well as 10.5, 15.75, and 20.05 mg/g dry weight, respectively (Supplementary Fig. S3). This result indicating that our recombinant bacteria could adsorb cadmium from wastewater containing other metals.

4. Discussion

As metal binding by biosorption is essentially a cell surface phenomenon (Misra et al., 2021), the role of Lpp-OmpA proteins in this process was assumed to be considerably important. Lpp-OmpA is an efficient display system developed by Georgiou et al. (1996) Lpp-OmpA has proven successful to transfer many types of proteins onto the bacterial surface. With this system, Tafakori et al. (2012) displayed bacterial MTs and a chitin-binding domain on the surface of *E. coli* for Cd binding, and Hui et al. (2018) expressed a putative Pb^{2+} binding domain (PbBD) derived from PbrR on *E. coli's* surface. We have demonstrated the potential use of our recombinant bacterial *E. coli* strains from an aqueous medium. The Cd^{2+}-chelating potency of our constructs (with an adsorption rate of 1.47 mg/g, equal to 13.077 nmol/mg) was comparable to those observed from some publications. For example, MTs were expressed on yeast and *E. coli* cell surfaces, respectively, with adsorption rate values of 27.1 and 14.9 nmol/mg dry weight (Kuroda and Ueda, 2006). Tafakori et al. (2012) also constructed the engineering *E. coli* with the expression of eukaryotic MTs or bacterial MTs on the bacterial surface, with adsorption rates of 2.8 nmol/mg dry weight or 13.5–26.4 nmol/mg dry weight, for Cd removal (Supplementary Table S5).

Langmuir's and Freundlich's models are commonly used to address biosorption kinetics for bacterial surface binding to heavy metals (Khan et al., 2016). In Langmuir's model (Eq. (1)), we assumed that the bacterial surface is uniform, and heavy metals would adsorb on the bacterial surface in a single layer homogeneously.

$$q_e = \frac{qmKLC_e}{1 + KLC_e}, \quad (1)$$

The values of q_e (mg/g) and q_m (mg/g) are the adsorption capacity and maximum adsorption capacity of our recombinant bacteria, respectively. C_e (mg/L) is the equilibrium concentration of Cd^{2+} in solution after biosorption, and K_L (L/mg) is Langmuir constant which can be obtained by calculation with previous parameters. However, Freundlich's isotherm model (Eq. (2)) describes multilayer adsorption on the surface of an adsorbent in a heterogeneous system whose available surface sites are not identical and differ in energies (Moshari et al., 2021).

$$q_e = K_F C_e^{1/n} \quad (2)$$

In Eq. (2), the definition of q_e and Ce is same as Eq. (1). K_F is the adsorption coefficient and *n* represents Freundlich constant. Table 1 shows the values of related parameters in Langmuir's and Freundlich's isotherm models. We found that higher regression coefficient values for Freundlich's isotherm model indicated that biosorption kinetics of our recombinant bacteria better fit Freundlich's model than Langmuir's model (Langmuir: $R^2 = 0.8568$; Freundlich: $R^2 = 0.9608$). However, Langmuir's model was considered unfavorable if separation factor (R_L) > 1 (Eq. (3)). In contrast, it was favorable when $0 < R_L < 1$ (Khan et al., 2016).

$$R_L = \frac{1}{1 + K_L C_0} \quad (3)$$

C_0 is the maximum concentration of Cd^{2+} in the solution (80 ppm) and K_L is the Langmuir constant (L/mg) form Eq. (1). In our data, we proved that R_L values fell within the range of $0 < R_L < 1$, indicating the feasibility of Langmuir's isotherm model for our recombinant bacteria.

We detected the characteristic peaks of many groups such as carboxyl, amino, phosphate groups and organic phosphate upon FT-IR analysis (Fig. 5). The shift of these peaks confirmed the binding of Cd^{2+} on the bacterial surface. The peak at 2922 cm^{-1} was due to alkyl chains (C–H stretching vibration) of fatty acids in phospholipid membranes (Ueshima et al., 2008). The peak at 1534 cm^{-1} was assigned to the linkage of amide, and moved to 1526 cm^{-1}, which resulted from Cd interaction with the amide group. The peaks of 1634 cm^{-1} and 1077 cm^{-1} indicated the existence of carbon- and phosphorous-containing oxygen atoms, and the moving of these peaks would suggest that these groups were interacted with Cd. Similarly, the shift of the peaks of the phosphate group, and organic phosphate was suggested to be involved in Cd interaction (Supplementary Table S6). Notably, the percentage of transmission peaks in treated bacteria was shifted from those of treated bacteria which indicated that stretching of bonds occurs after the Cd^{2+} binding on the bacterial surface, a result similar to Dong et al. (2019).

To understand whether Cd^{2+} biosorption on the surface of our recombinant bacteria affected resistance against Cd^{2+} toxicity, we tested the expression of six genes (*ftsZ, mutS, ef-tu, clpB, dnaK, and czcB*) with bacteria treated before and after Cd^{2+} (Khan et al., 2015). These genes were related to conferring the property to resist the toxicity of heavy metals among bacteria. The efflux of Cd^{2+} via energy-dependent efflux pumps provides effective resistance to Cd^{2+} toxicity (Khan et al., 2015; Han et al., 2022). As such, CzcCBA with three polypeptide chemiosmotic antiporters traverses from a bacterium's inner membrane to its outer membrane (Ndi and Barton, 2012). The *czcB* gene (1200 bp), one component of the *czcCBA* operon, was induced in pLO or pLM5 with Cd treatment, indicating that the ability of recombinant bacteria to efflux Cd^{2+} was enhanced. Furthermore, according to our results, MTT5 reduced the expression of the protein misfolding-related genes *dnaK* and *clpB* when bacterial cells treated with Cd^{2+}. The function of these genes is to capture misfolded proteins that Cd^{2+} injured and then fold them properly (Zolkiewski, 1999). These results indicated that bacteria harboring overexpression of intracellular or surface MTs could protect cells from Cd^{2+} cytotoxicity, reducing protein misfolding and avoiding Cd^{2+} accumulation in cytosol. In the presence of Cd^{2+}, the expression of the *mutS*, *ef-tu,* and *ftsZ* genes, play a role in genotoxicity, biosynthesizing new proteins and cellular replication, respectively, was not affected (Khan et al., 2015; Yan et al., 2021).

5. Conclusions

Our results showed that our recombinant bacteria harboring surface MTs could chelate with Cd by biosorption. FTIR analysis of the bacterial biomass revealed that some distinct function groups were involved in biosorption of Cd. The percentage of biosorption decreased along with increasing Cd concentrations. Cd adsorption by our recombinant bacteria's surface MT expression fit better with Freundlich's model than Langmuir's. In the presence of Cd, the recombinant bacteria were protected by surface MTs and were able to reduce the expression of some metal-resistance genes (i.e., *dnaK* and *clpB*). Our results supported our recombinant bacteria as an effective and low-cost biosorbent for Cd removal from aqueous solutions.

Table 1
Constants simulated with Langmuir and Freundlich models for Cd^{2+} biosorption using C43(DE3) pLysS expressing pLM5 as biosorbents.

Langmuir				Freundlich		
q_m	K_L	R^2	R_L	K_F	n	R^2
3.1308	0.0267	0.8568	0.3192	0.0677	1.0138	0.9608

q_m (mg/g): maximum adsorption capacity; K_L (L/mg): Langmuir constant; R_L: separation factor; K_F: adsorption coefficient; Freundlich constant.

Credit author statement

Che-Wei Lu: Validation, Investigation, Data curation, Writing – original draft, Visualization. **Hsin-Cheng Ho**: Investigation, Data curation. **Chao-Ling Yao**: Validation, Methodology, Resources. **Tsung-Yu Tseng**: Methodology, Resources. **Chih-Ming Kao**: Supervision, Project administration. **Ssu-Ching Chen**: Conceptualization, Resources, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.136850.

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