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Cadmium removal from aqueous solution by gene-modified *Escherichia coli* JM109

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Abstract

The article extended the study on the bioaccumulation of cadmium by genetically engineered bacterium *Escherichia coli* (namely M4) simultaneously expressing a cadmium transport system and metallothionein (MT). The growth of M4 showed resistance to the presence of cadmium. Compared with Cd^{2+} uptake capacity by original host bacterial cells, The Cd^{2+} accumulation of M4 was enhanced more than one-fold. M4 could effectively bind Cd^{2+} over a range of pH from 4 to 8. The presence of Ni^{2+} and Mn^{2+} did not influence Cd^{2+} uptake remarkably, but Cu^{2+}, Pb^{2+} and Zn^{2+} posed serious adverse effects. EDTA could drastically decrease Cd^{2+} bioaccumulation by M4, whereas the effect of citrate was relatively slight.

Keywords: Cadmium; Genetically engineered *Escherichia coli*; Bioaccumulation; Heavy metal wastewater

1. Introduction

Cadmium has been widely used in electroplating, smelting, alloy manufacturing, pigment, plastic, battery, mining and refining processes, which might lead to cadmium releasing into the environment by waste streams [1,2]. Solid wastes are disposed of in landfill sites, resulting in large cadmium inputs at the national and regional levels. The application of phosphate fertilizers or municipal sewage sludge fertilizer in agriculture represents a direct input of cadmium into soils and can be a significant source of the cadmium taken up by plants [3]. Due to the increasing value of some metals such as cadmium, as well as due to a greater awareness of the ecological effects of toxic metals released into the environment, studies of metal accumulation have been carried out on the removal and recovery of metals. Among those methods, using natural materials of biological origin has been focused in recent years [4–6]. However, current technologies for cadmium removal from wastewater such as biosorption (mainly employing inactivated/dead biomass) lack a sufficiently high affinity and selectivity to reduce residual cadmium to the levels dictated by ever more stringent government regulations [7].

In our previous studies, genetically engineered bacteria with both high bioaccumulation capacity and high affinity to desired metals such as mercury and nickel had been successfully constructed, and the experimental results showed the recombinant bacterial cells could selectively accumulate the goal metal ions from multi-component solutions under varying environmental conditions [8,9]. These results suggested the application of genetic engineering technique in the treatment of heavy metal wastewater would be an intriguing alternative to biosorption. Two Cd^{2+} uptake genes, namely *mntA* and *cdtB*, respectively, were cloned from *Lactobacillus plantarum* in Wilson Laboratory [10]. MntA is a high-affinity Mn^{2+} and Cd^{2+} transport system that can only be induced by Mn^{2+} starvation, while CdtB is a low-affinity Cd^{2+} transport system able to be expressed both in Mn^{2+}-starve and Mn^{2+}-sufficient cells. The finding of cadmium transport system made it possible to construct a Cd^{2+}-accumulating strain. In this paper, therefore, genetically engineered bacteria were constructed to confer on the cells with the capability of specifically accumulating Cd^{2+} from aqueous solution by expressing cadmium transport system and metallothionein (MT). Considering Mn^{2+} commonly exists in aqueous environment, especially industrial heavy metal wastewater,

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CdtB was chosen in this study as Cd^{2+} transport system. MTs are a class of low-molecular-weight proteins rich in cysteine residues, therefore the expression of MT can enable the cells to bind more heavy metal ions.

2. Materials and methods

2.1. Plasmids and strains

Two compatible plasmids, pCLG2 (Deng et al., unpublished) and pGPMT [11], which expressed CdtB and the GST fusion protein of pea MT (GST-MT), respectively were employed in this study. To construct plasmid pCLG2, plasmid pCL2-29L [10] which contained the entire *cdtB* gene was digested with the restriction enzymes *Sal*I and *Kpn*I, and the resultant 2.3 kb fragment containing *cdtB* was inserted and ligated between the *Sal*I and *Kpn*I cloning sites of pCL1921. pCLG2 contained Spc^r sequence, and pGPMT contained Amp^r sequence. The host strain used in this study was *Escherichia coli* JM109.

The recombinant *E. coli* cells expressing cadmium transport system and/or MT were cultured overnight at 37 °C in Luria–Bertani (LB) broth supplemented with spectinomycin (30 mg/L) and/or ampicillin (50 mg/L), diluted to 0.1–0.3 (OD_{600}) with the same fresh media, and then incubated at 37 °C with vigorous shaking (180 r/min). When the OD_{600} reached 0.5–0.7, isopropyl β-D-thiogalactoside (IPTG) (1 mM) was added. Cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4 °C after 4 h of additional cultivation. Original host *E. coli* JM109 cells were also prepared as above to be the control in bioaccumulation experiment, except for the antibiotics adding and IPTG induction.

2.2. Cd^{2+} bioaccumulation

Cultured cells were harvested by centrifugation, washed three times with 10 mM phosphate buffer (pH 7.0) and resuspended in the solutions with the desired Cd^{2+} concentrations. After a 1 h incubation at 180 r/min and 37 °C, the cells were separated by centrifugation, dried, and digested overnight with 70% trace-metal grade nitric acid for cadmium analysis.

The influences of other environmental factors were tested as follows. *E. coli* cells were suspended in 50 mg/L Cd^{2+} solution containing Cu^{2+}, Pb^{2+}, Hg^{2+}, Ni^{2+} or Mn^{2+} with the concentration in the range of 0–1000 mg/L, respectively. For pH, 0.1 M NaOH or HNO_3 was used to adjust the suspension to the desired value. EDTA and sodium citrate were added to evaluate the effect of chelators. During these experiments, the stirred speed and temperature were kept constant at 180 r/min and 37 °C, respectively, unless otherwise stated. All treatments were in triplicate.

2.3. Analysis

An atomic absorption spectrophotometry (Hitachi Z-8200) was used to determine the concentration of Cd^{2+} in the solution or cells. The dry weight of cells was measured from the OD_{600} by using the value of 0.465 g dry weight per liter of OD1.0. Cadmium binding capacity was expressed as milligram Cd^{2+} accumulated by gram dry weight of cells.

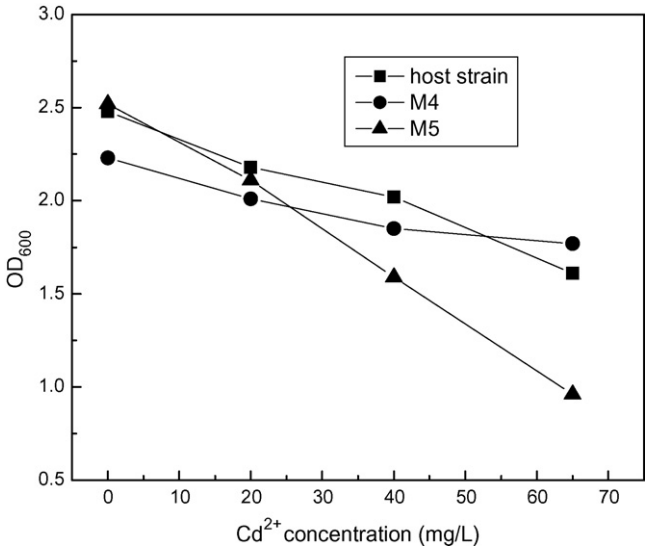

Fig. 1. Effect of Cd^{2+} on the growth of *Escherichia coli* JM109 containing various plasmids in LB. M4 harbored both pCLG2 and pGPMT, M5 harbored pCLG2 only.

3. Results and discussions

3.1. Resistance of recombinant strains to Cd^{2+}

E. coli JM109 strains containing pCLG2 and pGPMT (namely M4) or pCLG2 only (namely M5) were grown in LB + Cd^{2+} solutions to test their sensitivities to the presence of Cd^{2+}. The original host *E. coli* JM109 cells were also grown as the control. After 16 h incubation at 37 °C for each culture, the OD_{600} data were detected and displayed in Fig. 1. Among three tested strains, M5, which only harbored pCLG2 to express CdtB protein, was more sensitive to Cd^{2+} than original host strain, whose OD_{600} dropped 62% from 2.52 in LB to 0.96 in LB + 65 mg/L Cd^{2+}. On the other hand, by simultaneously expressing cadmium transport system and MT, M4 showed a strong resistance to the presence of Cd^{2+}, with its OD_{600} only declining 20% as Cd^{2+} concentration in the medium increased from nil to 65 mg/L. The fact that M5 was super-sensitive to Cd^{2+} suggested more Cd^{2+} ions were transported into the cells by cadmium transport system and thus resulted in serious toxic effect. However, this toxic effect could be effectively weakened by simultaneously expressing MT in cytoplasm. Therefore, both *cdtB* and MT gene were needed to construct a Cd^{2+}-resistant strain.

3.2. Bioaccumulation capacity of Cd^{2+} by genetically engineering strains

Fig. 2 shows bioaccumulation capacity of Cd^{2+} by three tested strains after 1 h incubation. In comparison with 30.2 mg/g by host cells at initial Cd^{2+} concentration of 60 mg/L, the Cd^{2+}-accumulating capacity of M4 and M5 were enhanced to 63.26

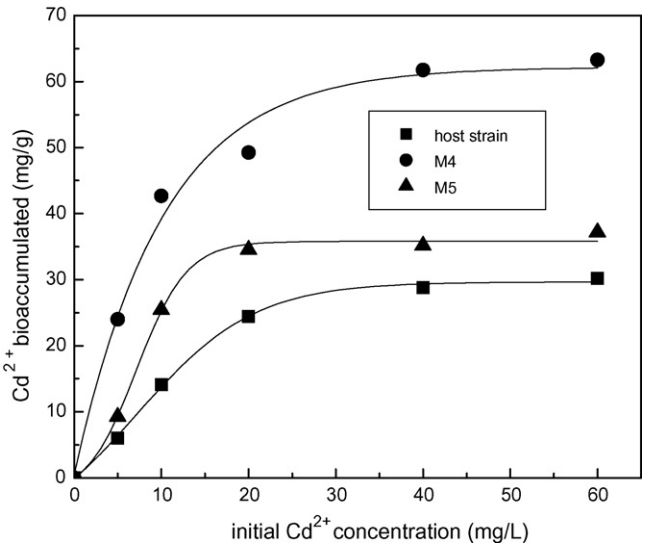

Fig. 2. Cd^{2+} bioaccumulation capacity of *E. coli* JM109 containing various plasmids. M4 harbored both pCLG2 and pGPMT, M5 harbored pCLG2 only.

and 37.16 mg/g, respectively. It could be seen that M5 only bound a little more Cd^{2+} than host strain, whereas simultaneous expression of CdtB and MT conferred on M4 with more than one-fold increase of Cd^{2+}-binding capacity, indicating MT was important to improve metal binding capacity of the recombinant cells. Accordingly, the Cd^{2+} transport system enabled the cells to bind and transport Cd^{2+}, and intracellular overexpression of MT allowed the recombinant cells to capture more Cd^{2+}.

In the previous study, a genetically engineered strain which simultaneously expressed nickel transport system and MT could bind six times of Ni^{2+} as much as host strain [8]. By simultaneously expressing pKNA and pGPMT in *E. coli* JM109, Krishnaswamy obtained a recombinant strain with a four-fold-higher Ni^{2+} accumulation [12]. A recombinant *E. coli* harboring both mercury transport system and MT also received a four-fold increase of Hg^{2+} uptake capacity compared to the control [13]. In another work, Kim used *mntA*, which encoded another cadmium transport system, and MT gene to construct a Cd^{2+}-accumulating recombinant strain [14]. A six-fold increase of Cd^{2+} uptake capacity was obtained compared to the control in that work. However, only one-fold increase of metal bind capacity was obtained after similar gene operation in this work. It might be because the CdtB, a low-affinity Cd^{2+}-transporter compared to MntA, could only accumulate Cd^{2+} on a relatively low level in comparison to other studies.

A summary of various biomasses used for Cd^{2+} uptake in previous studies was shown in Table 1. Although direct comparison of the strain used in our study with other reported biomasses was difficult due to the varying experimental conditions, the bioaccumulation capacity of our recombinant *E. coli* was relatively high, indicating promising potential for Cd^{2+} removal.

3.3. Effect of pH

Metal sorption is critically linked with pH. For surface adsorption like biosorption process, availability of negatively charged groups at the biosorbent surface is necessary for the sorption of metals to proceed [15]. Lopez indicated that the pH of the solution had a marked effect on the metal biosorption by *P. fluorescens*. A variation of pH from 8 to 6.5 led to the decrease of metal biosorption up to 90% [16].

In our study, within the pH range from 4 to 8, M4 could retain more than 27.5 mg/g of Cd^{2+} bioaccumulation with the maximum reduction being 35% at pH 4.0, whereas original host *E. coli* cells showed a 63% decrease of Cd^{2+} adsorption capacity (Fig. 3). The resistance of M4 to pH variation might be attributable to the expression of CdtB, which made Cd^{2+} be transported into the cells, so the bioaccumulation process was less linked with the situation of cell surface, and consequently less sensitive to pH change. This result was in consistent with the previous work [17].

Table 1
Maximum cadmium(II) uptake capacities of some reported biomasses

Biomass	q_{max} (mg/g)	References/ source
Streptomyces noursei	6.74	[1]
Rhizopus arrhizus	49.46	[2]
Chitin	14.0	[4]
Sargassum sp.	120	[5]
Cicer arientinum	38.76	[6]
Padina sp.	59.57	[19]
Streptomyces albus HUT 6047	30.35	[21]
Recombinant *Escherichia coli* (pCLG2 + pGPMT)	63.26	This study

3.4. Effect of co-existing metals on Cd^{2+} bioaccumulation

It is known nickel ion is a recalcitrant pollutant and many metal tolerant microorganisms have a relatively low Ni^{2+}-binding capacity [18]. However, cadmium has a similar characteristic with nickel at this aspect. Although previous stud-

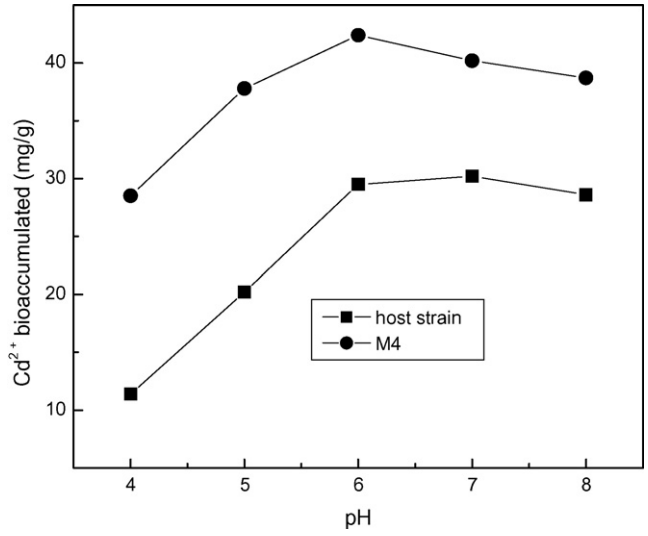

Fig. 3. Effect of pH on Cd^{2+} bioaccumulation by *E. coli* cells from 50 mg/L Cd^{2+} solution.

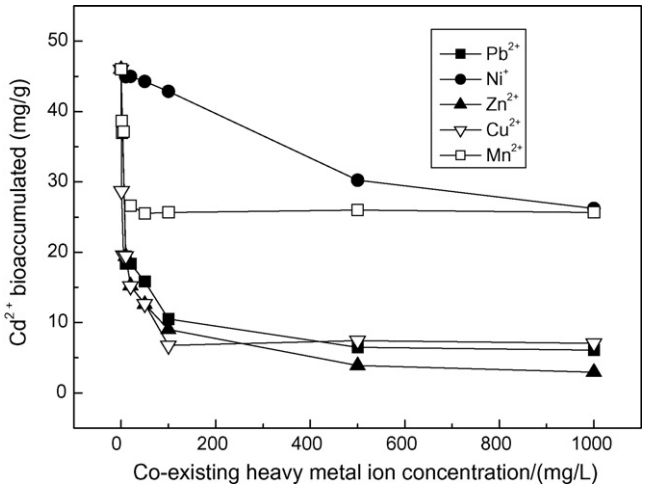

Fig. 4. Effect of co-existing metal ions on Cd^{2+} bioaccumulation by M4 from 50 mg/L Cd^{2+} solution containing other heavy metals, respectively.

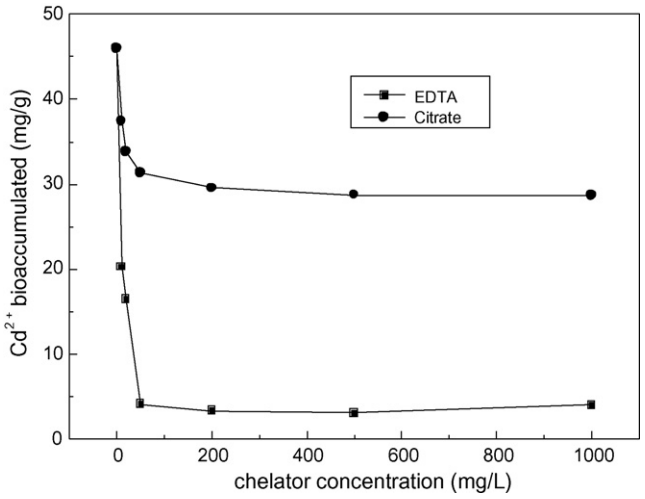

Fig. 5. Effect of chelators on Cd^{2+} bioaccumulation by M4 from 50 mg/L Cd^{2+} solution.

ies reported high Cd^{2+}-binding capacities of some biosorbents [19,20], these results were obtained by applying biosorbents into aqueous solutions containing only Cd^{2+}. Nakajama evaluated the selective heavy metal accumulation of 83 microorganisms from mixed metals solution containing 40 μM Cd^{2+} (4.5 mg/L) and eight other heavy metal ions [21]. The quantity of Cd^{2+} absorbed by almost all tested species was found to be extremely low, much less than copper, lead and mercury, indicating the biosorption of cadmium was easily interfered by the presence of other metal ions. To investigate the selective accumulation of Cd^{2+} over other co-existing metal ions by genetically engineered *E. coli*, bioaccumulation was performed in the presence of various concentrations of Pb^{2+}, Ni^{2+}, Cu^{2+} and Mn^{2+}, respectively. From Fig. 4 it could be deduced that Ni^{2+} and Mn^{2+} did not affect Cd^{2+} uptake significantly. Almost 60% of Cd^{2+}-binding capacity of M4 was retained with the concentration of Ni^{2+} and Mn^{2+} coming up to 1000 mg/L, 20 folds of initial Cd^{2+} concentration. Pb^{2+} and Cu^{2+}, however, inhibited Cd^{2+} uptake process more seriously. As the concentration of Pb^{2+} and Cu^{2+} reached 50 mg/L, Cd^{2+} uptake capacity of M4 decreased 67% and 74%, respectively. Surprisingly, Zn^{2+}, whose toxic effect was weaker than Pb^{2+} and Cu^{2+}, posed a severely adverse effect on Cd^{2+} accumulation by M4. Only 8% of Cd^{2+} uptake capacity of M4 was remained as Zn^{2+} concentration was 500 mg/L. The inhibition effect of Zn^{2+} might be partly based on the fact that Zn^{2+} and Cd^{2+} are in the same group of the element periodical table, thus the similar atomic structure results in a possible competitive binding with CdtB protein between these two metals ions. On the other hand, it was reported that *E. coli* cells possess a constitutive Zn^{2+} transport system by which Cd^{2+} can enter the cells at the lack of Zn^{2+} in the environment [22]. Therefore, it could be deducted that the presence of Zn^{2+} might not only prevent Cd^{2+} from entering the cells via Zn^{2+} transport system, but also inhibit Cd^{2+} uptake through cadmium transport system expressed by *cdtB* gene in M4.

In Kim's study, Cd^{2+} bioaccumulation of genetically engineered *E. coli* simultaneously expressing MntA protein and MT was seriously inhibited by the presence of Mn^{2+} [14]. Cd^{2+} accumulation was significantly decreased to 20% of that of the control by the addition of Mn^{2+}. The reduction of Cd^{2+} accumulation might be because MntA, a high-affinity Mn^{2+} uptake system that also effectively took up Cd^{2+}, could only be induced by Mn^{2+} starvation as it was originally found in *L. plantarum* [23]. Therefore, the presence of Mn^{2+} might inhibit the transport of Cd^{2+} by MntA. CdtB, however, originally expressed in Mn^{2+}-sufficient cells [23], would not be affected by the presence of Mn^{2+}.

3.5. Effect of chelators on Cd^{2+} bioaccumulation

Chelators could form tight complexes with most heavy metals and consequently decrease their bioavailability by biosorption. Two common chelators, EDTA and citrate, were tested for their influences on Cd^{2+} bioaccumulation by genetically engineered bacteria. The result (Fig. 5) indicated that the presence of EDTA posed a severe deleterious effect on Cd^{2+} uptake. As EDTA concentration reached 50 mg/L, Cd^{2+} uptake capacity by M4 dropped to 4.16 mg/L, meaning 90% reduction. On the contrary, the effect of citrate was relatively slight. Almost 60% of Cd^{2+}-binding capacity was retained with the concentration of citrate up to 500 mg/L, indicating Cd^{2+} bioaccumulation process by the recombinant cells was resistant to the presence of citrate. The different effect of EDTA and citrate on Cd^{2+} uptake by M4 could be explained that EDTA has a stronger ability to form complex with transition metals than citrate, thus easily making more free Cd^{2+} ions be turned into Cd^{2+}-complex which could not be recognized by Cd^{2+} transport system, consequently decreasing Cd^{2+} bioaccumulation by recombinant strain to a larger extent. Furthermore, EDTA was found very harmful to the growth of microorganism cells [24]. This harmful effect might inhibit certain activity of live cells related to metal uptake. Citrate, however, as one of the metabolites of microorganism cells, will not pose seriously deleterious effect on the physiological condition of the cells, so no serious inhibition will be produced.

4. Conclusions

Genetically engineered *E. coli* JM109, which simultaneously expressed a cadmium transport system and MT (namely M4) was evaluated for its ability to accumulate Cd^{2+} from aqueous solutions. M4 showed resistance to the toxicity of cadmium and could accumulate 63.26 mg/g Cd^{2+}, which is more than one times of Cd^{2+} uptake capacity of original host strain. During the tested pH range (4–8), the Cd^{2+} uptake process by M4 was resistant to pH variation. The presence of Cu^{2+}, Pb^{2+} and Zn^{2+} severely inhibited Cd^{2+} uptake by M4, whereas Ni^{2+} and Mn^{2+} gently decrease Cd^{2+} bioaccumulation. EDTA was very harmful to Cd^{2+} binding, but the adverse effect of citrate was insignificant.

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