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Metal biosorption capability of *Cupriavidus taiwanensis* and its effects on heavy metal removal by nodulated *Mimosa pudica*

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Abstract

A novel metal biosorption system consisting of the symbiotic combination of an indigenous metal-resistant rhizobial strain, *Cupriavidus taiwanensis* TJ208, and its host plant *Mimosa pudica* has been developed for the removal of heavy-metal pollutants. Free-living *C. taiwanensis* TJ208 cells were able to adsorb 50.1, 19.0, and 19.6 mg/g of Pb, Cu, and Cd, respectively. After nodulation via inoculation with strain TJ208, the metal uptake ability of *M. pudica* markedly increased, as the nodulated *M. pudica* displayed a high metal uptake capacity (q_{max}) of 485, 25, and 43 mg/g, respectively, which is 86, 12, and 70% higher than that of nodule-free plants. Moreover, with TJ208 nodules, the *M. pudica* plant also displayed a 71, 81, and 33% enhancement in metal adsorption efficiency (η) for Pb, Cu, and Cd, respectively. The nodulation appeared to give the greatest enhancing effect on the uptake of Pb, which is consistent with the preference of metal adsorption ability of TJ208. This seems to indicate the crucial role that the rhizobial strain may play in stimulating metal uptake of the nodulated plant. Furthermore, the results show that metal accumulation in the nodulated plant mainly occurred in the roots, accounting for 65–95% of total metal uptake. In contrast, the nodules and the shoots only contributed to 3–12 and 2–23% of total metal uptake, respectively. Nevertheless, the specific adsorption capacity of nodules is comparable to that of the roots. Hence, this work demonstrates the feasibility and effectiveness of using the nodulated plants to promote phyto-removal of heavy metals from the polluted environment as well as to restrict the metal contaminants in the unharmful region of the plant.

Keywords: Biosorption; *Cupriavidus taiwanensis*; Heavy metals; *Mimosa pudica*; Nodulation; Rhizobium

1. Introduction

Heavy-metal pollution in aquatic and soil environments has become a common issue for both developing and developed countries. Heavy metals have been intensively utilized in a variety of industries and are of great environmental concern because of their severe toxicity and unique characteristics. Unlike organic pollutants, heavy metals are non-biodegradable and tend to accumulate and concentrate in living organisms via the food chain [1]. Problems arising from heavy-metal pollution are major threats to human health and to the entire ecosystem. To create a world that can both drive robust industrial growth and to sustain environmental demands, technologies leading to clean-up of heavy-metal pollution are of increasing demand.

A range of methods has been used for heavy metal removal, and one of the most common methods is adsorption. In particular, using natural and environmentally compatible materials (e.g., biomass of microorganisms and plants) for metal adsorption (called biosorption) is of great interest [2–4]. Microorganisms and plants with the ability to resist, detoxify, and adsorb metals have been widely studied for their potential to remediate metal-contaminated environments [5,6]. Developing efficient biosorbents and biosorption systems appears to be one of the solutions toward bioremediation of environments contaminated by heavy metals in an effective, natural, and economically feasible way [5–7].

Rhizobia grow slowly for long periods in soil, but if they infect a compatible legume they can grow rapidly; successful

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infection by a single bacterium can lead to the formation of a nitrogen-fixing nodule on the root of legume, containing over 10^8 bacterial progeny [8]. There have been reports describing metal-resistant rhizobia [9–12]. Recently, genetically engineered rhizobia systems were developed to carry out bioremediation for heavy metals [13–16]. A novel root nodule bacterium, *Cupriavidus taiwanensis* (formerly named as *Ralstonia taiwanensis*), was recently isolated from *Mimosa* spp. in Taiwan [17–20], and it has been confirmed that *C. taiwanensis* is capable of nodulation and nitrogen fixation in their host plants [18,19]. *C. taiwanenesis*, together with other newly described β-proteobacteria rhizobia, particularly *Burkholderia* spp., are different from the well-known rhizobia of the family Rhizobiaceae in the α-proteobacteria [18,19,21]. The genus *Cupriavidus* is of particular biotechnological interest due to its great biodegradation capability for recalcitrant compounds and xenobiotics [22,23]. Moreover, some environmental isolates of *Cupriavidus* species, such as *C. necator* (formerly named as *Ralstonia eutropha* or *Alcaligenes eutrophus*) and *C. metallidurans* are also well-known metal-resistant bacteria [24-26]. These *Cupriavidus* species were previously demonstrated to have detoxification pathways for a broad range of metals, as they bear megaplasmids controlling resistance against Cd, Co, Zn, Cu, Pb, Ni, Hg, and Cr [26].

In light of potential applications of the novel indigenous root nodule bacterium *C. taiwanensis* in heavy-metal treatment, this study was undertaken to explore in detail its heavy-metal resistance and removal capability. In addition, a novel biosorption system using the symbiotic combination of *C. taiwanensis* and its host plant *Mimosa pudica* is proposed, as plants are also frequently used to uptake heavy metals from the environment. Due to the poor growth and low tolerance of plants whilst being exposed to heavy metals, many previous studies have utilized harvested and pretreated plant biomass instead of live plants as the biosorbent for metal removel [27,28]. However, for *in situ* decontamination of heavy metals, it would be more desirable to use live plants and, therefore, in the present study, the metal uptake abilities of the rhizobial strain, *C. taiwanensis* TJ208, and its host plant, *M. pudica*, with or without root nodules were compared. Three common metal contaminants (i.e., Pb, Cu, and Cd) in the industrial effluents in Taiwan were chosen as model metal adsorbates to assess the effectiveness and feasibility of using the nodulated plants as a biosorbent for the clean-up of metal pollution in the environment.

2. Materials and methods

2.1. Bacterial strains and culture conditions

A total of 200 strains of *C. taiwanensis* originally isolated from root nodules of *M. pudica*, *M. diplotricha* or *M. pigra* were tested for their heavy-metal resistance [17–19,29,30]. These strains were identified as *C. taiwanensis* by 16S rDNA PCR-RFLP, *nifH* PCR-RFLP, *nodA* PCRRFLP and 16S rDNA sequences [17–19,29].

2.2. Determination of heavy-metal resistance and minimum inhibitory concentration

The broth method of heavy-metal-resistance testing was accomplished using LB broth amended with one heavy metal at one concentration per culture. The range of metal concentrations that was examined was 100–1500 mg l^{-1} of Pb^{2+}, Cu^{2+}, and Cd^{2+}. Each heavy-metal-amended LB broth (5 ml) was inoculated with 1×10^8 of *C. taiwanensis* cells, and was then cultured for 72 h at 35 °C and 200 rpm agitation. Following cultivation, the optical density at 600 nm (OD_{600}) was measured and recorded. A culture with an OD_{600} of greater than 0.5 was considered resistant.

The method used to determine minimum inhibitory concentration (MIC) was adapted from the spread-plate method described by Mergeay et al. [31]. The medium used for MIC measurement was LB agar medium supplemented by a specific heavy-metal salt. The agar plates containing one concentration of either $Pb(NO_3)_2$, $CuCl_2$, $ZnCl_2$, $CoCl_2$, $NiCl_2$ or $CdCl_2$ were prepared (all obtained from Sigma, Taiwan). The following metal concentrations used were 2.5, 5.0, 7.5, 10, 12.5, and 15 mM. The MIC was defined as the lowest concentration at which no viable colony-forming units (CFU) were observed after 5 d incubation at 35 °C.

2.3. Procedures of metal biosorption by C. taiwanensis TJ208

2.3.1. Preparation of the bacterial biosorbent

The *C. taiwanensis* TJ208 strain was precultured with LB broth at 37 °C for 24 h, until early stationary phase was reached. After centrifugation at 9000 rpm for 10 min, the supernatant was removed, and the cell pellet was rinsed with deionized water two to three times prior to being used for biosorption experiments.

2.3.2. Time-course biosorption profile

The prepared bacterial biosorbent was suspended in metal solutions (100 ml each) containing 100 mg l^{-1} of Pb, Cu, or Cd. The biomass concentration in the suspension was controlled at *ca*. 1.0 g l^{-1}. The pH of metal solutions was adjusted to 5.0 for Pb and Cu, and 6.0 for Cd by Tris buffer to avoid metal precipitation due to formation of metal hydroxides [32]. The cell-metal suspension was incubated at 37 °C with gentle agitation (100 rpm). Samples were taken at designated time intervals and were then centrifuged at 13,000 rpm for 5 min. The supernatant was subjected to analysis by atomic absorption spectrometry (AAS) with a Polarized Zeeman Atomic Absorption Spectrometer (Hitachi Model-Z-6100, Tokyo, Japan) to determine the residual metal concentration (C_e; mg l^{-1}). The capacity of biosorption (q; mg metal g dry cell^{-1}) was calculated by $q = (C_0 - C_e)/X$, where C_0 is the initial metal concentration (mg l^{-1}) and X is the biomass concentration (g dry cell l^{-1}).

2.3.3. Biosorption isotherm

To determine the biosorption equilibrium of each metal, adsorption isotherm experiments were conducted. Similar to the procedures described earlier, the biosorbent (1 g l^{-1}) was added to metal solutions containing different concentrations of each metal ion. The residual metal concentration in the suspensions was measured after 24 h incubation at 37 °C and 100 rpm agitation. The experimental results were simulated by Langmuir isotherm ($q = q_{max} C_e/(K_d + C_e)$) to determine the characteristic parameters (q_{max} and K_d) of biosorption, where q_{max} denotes maximum adsorption capacity (mg g dry cell^{-1}), and K_d denotes the Langmuir constant (mg l^{-1}).

2.4. Plant cultivation

Plant cultivation and nodulation tests were carried out as described by Chen et al. [18,19] with some modification. *M. pudica* seeds were surface sterilized with concentrated sulfuric acid for 10 min, followed by treatment with 3% sodium hypochloride for 10 min. The sterile seeds were germinated on 1% water agar at 28 °C in darkness. Plant cultivation was carried out using the tube method of Gibson [33]. The tube contained a modified Jensens nitrogen-free plant nutrient medium (pH fixed at 6.8–7.0) [34] and was incubated at 35 °C under an irradiation of 400 μmol m^{-2} s^{-1} and a photo period of 16 h. Seven days after germination, seedlings were inoculated with 100 μl (*ca.* 10^5 cells) suspension of *C taiwanensis*. In the control run (without inoculation), 50 mg l^{-1} KNO_3 was amended to the culture tube to support plant growth, and the culture pH was controlled at 6.8–7.0. Plants were harvested for 21 d after inoculation. The dry weight of nodulated and nodule-free plants was controlled at a similar range to avoid the effect of biomass loading.

2.5. Procedures of metal adsorption by plants

Four 3-week-old nodulated and non-nodulated *M. pudica* plants were transferred to 20 ml solutions containing metal ions of Pb, Cu, and Cd at a concentration of 50–400 mg l^{-1}. After incubation for 7 d, the plants were taken from the solutions. The root, nodule, and shoot portions were separated and the dry weight of each portion was measured. To determine the metal uptake in different parts of the plant, the roots, nodules, and stems/leaves were ashed separately at 450 °C for 12 h, and the resulting ashes from each plant part were extracted with 10 ml of an acid solution containing 6N HCl and 3N HNO_3. The heavy-metal concentration in the acid extracts was measured by AAS. The adsorption capacity at each initial metal concentration in each part of the plants was then calculated. The results were also simulated by Langmuir isotherm as described earlier.

2.6. Measurement of heavy metals

The metal salts used in this work were $Pb(NO_3)_2$, $CuCl_2$, $ZnCl_2$, $CoCl_2$, $NiCl_2$ and $CdCl_2$. All of them were obtained from Sigma. Heavy-metal concentration in the solution was measured with a Polarized Zeeman Atomic Absorption Spectrometer (Hitachi Model-Z-6100, Tokyo, Japan).

3. Results

3.1. Metal resistance of C. taiwanensis strains

Primary screening for heavy-metal resistance was conducted on our collection of 200 strains of *C. taiwanensis* isolated from root nodules of *M. pudica*, *M. diplotricha*, and *M. pigra* in Taiwan. Of the 200 isolates, strain TJ208 was selected for further studies due to its higher heavy-metal resistance than the other strains. Strain TJ208 can grow in LB amended with up to 1500, 700, 300, 250, or 200 mg l^{-1} of Pb, Zn, Cu, Ni, and Cd, respectively. In contrast, the type strain of *C. taiwanensis*, LMG 19424, can only grow in LB amended with 1000, 500, 200, 150, or 100 mg l^{-1} of Pb, Zn, Cu, Ni, and Cd. The MIC of strain TJ208 to various heavy metals was also determined. As indicated in Table 1, MIC values of strain TJ208 for Pb, Zn, Co, Cu, Cd, and Ni were 15, 7.5, 5, 5, 2.5, and 1.5 mM, respectively. These MIC values are much higher than those of a standard *Escherichia coli* strain but were quite similar to those for a well-known metal-resistant strain, *C. metallidurans* CH34 [31,35]. In addition, the MIC of the *Cupriavidus* strain and the *R. eutropha* strain had similar dependence on heavy metals (Table 1).

3.2. Metal biosorption with C. taiwanensis TJ208

The time-course biosorption profiles of *C. taiwanensis* TJ208 for Pb, Cu, and Cd are illustrated in Fig. 1, and it shows that with an initial metal concentration of 100 mg l^{-1} the biosorption equilibrium was nearly reached within 200–400 min. The initial adsorption rate (r_{ads}), calculated according to Eq. (1), was 0.81, 0.55, and 0.38 mg g cell^{-1} min^{-1} for Pb, Cu, and Cd, respectively, indicating that the TJ208 strain had a higher adsorption rate for Pb, followed by Cu and Cd:

$$r_{ads} = -\frac{1}{X}\left.\frac{dC}{dt}\right|_{t\to 0} = \left.\frac{dq}{dt}\right|_{t\to 0} \quad (1)$$

Furthermore, the results of biosorption isotherms can be precisely described by a Langmuir isotherm model with high r^2

Table 1
Comparison of the minimum inhibitory concentration (MIC) of *Cupriavidus taiwanensis* TJ208, *Ralstonia eutropha* CH34, and *Escherichia coli* for various heavy metals

Strain	MIC (mM)						References
	Pb	Cu	Cd	Zn	Co	Ni	
R. eutropha CH34	NA	3.0	2.5	12	5.0	2.5	[31]
C. taiwanesis TJ208	15	5.0	2.5	7.5	5.0	1.5	This work
E. coli	0.2	1.0	0.5	1.0	1.0	1.0	[35]

NA: not available.

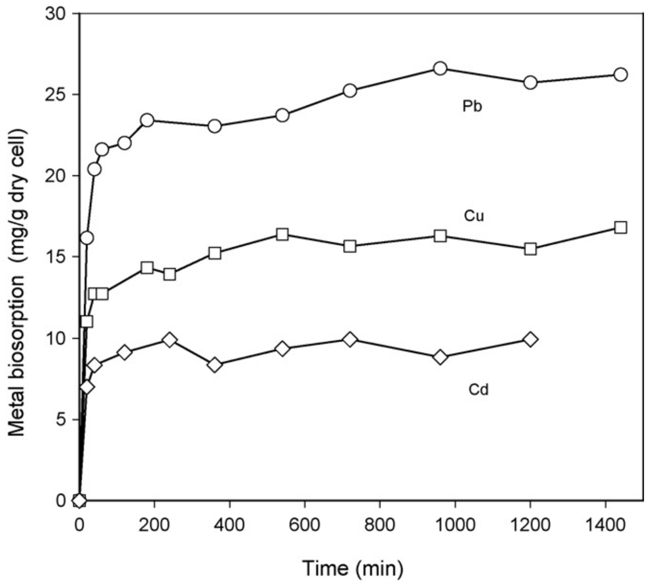


Fig. 1. Time-course adsorption profiles for Pb, Cu, and Cd ions by *Cupriavidus taiwanensis* TJ208 (initial metal concentration = 100 mg l^{-1}).

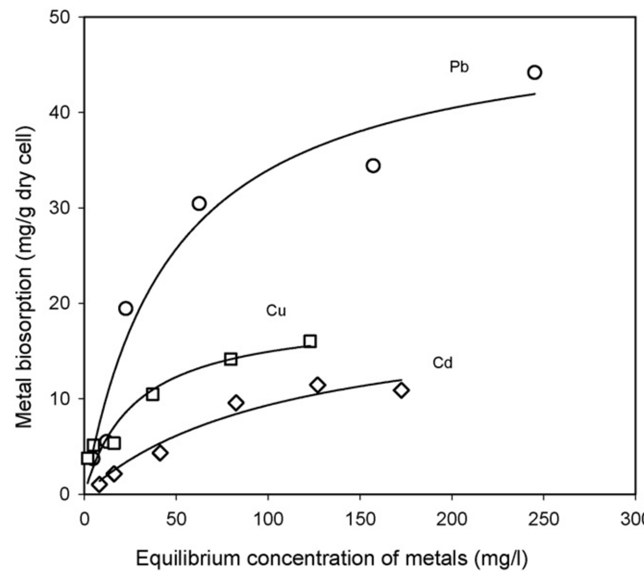


Fig. 2. Adsorption isotherms of Pb, Cu, and Cd by *C. taiwanensis* TJ208 (symbols: experimental data; curves: simulation with Langmuir isotherm).

values (Table 2 and Fig. 2). The estimated maximum adsorption capacity (q_{max}) was 50.1, 19.0, and 19.6 mg g $cell^{-1}$ for Pb, Cu, Cd, respectively, while Cu biosorption displayed the lowest K_d value of 27.5 mg l^{-1}, followed by Pb (47.4 mg l^{-1}) and then Cd (59.8 mg l^{-1}) (Table 2).

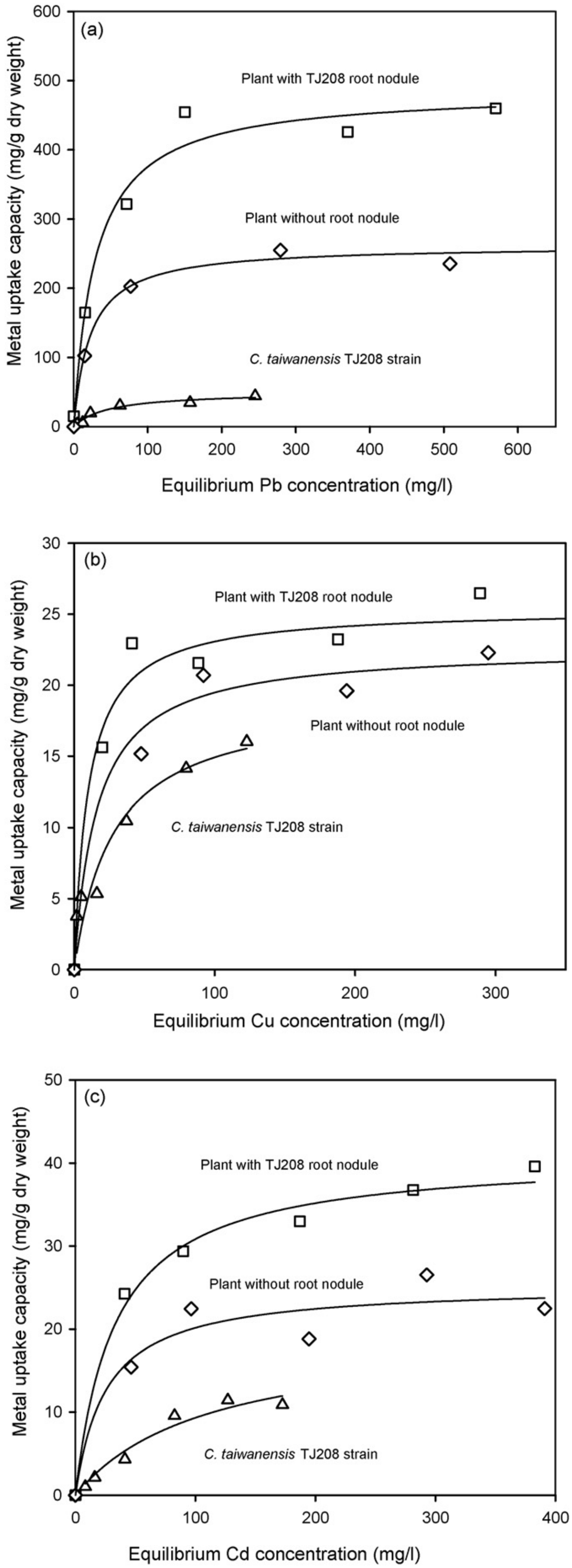
3.3. Metal biosorption by M. pudica with or without TJ208 nodules

M. pudica plants inoculated with TJ208 were cultivated in nitrogen-free plant nutrient medium for 3 weeks. The average dry weight of inoculated plants after 3 weeks of cultivation was 11.91 ± 2.41 mg per plant. The average number of root nodules on inoculated plants was 5.98 ± 1.87 root nodules per plant. The control plants (without TJ208 inoculation) were cultivated with the same nutrient medium as used for the inoculated plants, except that 50 ppm KNO_3 was supplemented to help plant growth. Over the same cultivation period the uninoculated control plants gained similar dry weights (12.33 ± 1.81 mg per plant) to inoculated plants (11.91 ± 2.41 mg per plant), thus suggesting that the different growth conditions used for inoculated and nodule-free plants allowed a proper control of plant growth, resulting in a similar biomass yield for both sets of plants. Therefore, as metal biosorption was conducted with plants having similar weights, the effect of biomass loading on biosorption can be excluded. Metal biosorption isotherms of the nodulated plants, control plants, and the TJ208 strain are compared in Fig. 3. The simulation results are also listed in Table 2. The results show that regardless of the metal adsorbates used, the plants with TJ208 nodules had a higher adsorption capacity than the nodule-free control plants, whilst the adsorption capacity of the free-living strain TJ208 was always lower than the plants (with or without nodules). Nodulation after inoculation with TJ208 markedly increased the metal uptake capacity of the *Mimosa* plants, leading to 86, 12, and 70% increases in equilibrium capacity of Pb, Cu, and Cd, respectively (Table 2).

Table 2
Adsorption equilibrium and Langmuir isotherm simulations for biosorption of Pb, Cu, and Cd by *C. taiwanensis* TJ208, *Mimosa pudica*, and *M. pudica* with TJ208 nodules

Metal adsorbate	Biosorbent[a]	Estimated parameters for Langmuir isotherm			Adsorption efficiency (η)[b]
		K_d (mg metal/l)	q_{max} (mg metal/g dry weight)	r^2	
Pb	TJ208	47.4	50.1	0.956	1.06
	M. pudica inoculated with TJ208	26.1	485	0.988	18.5
	M. pudica without inoculation	24.2	261	0.977	10.8
Cu	TJ208	27.5	19.0	0.887	0.69
	M. pudica inoculated with TJ208	10.1	25.4	0.966	2.51
	M. pudica without inoculation	16.3	22.7	0.923	1.39
Cd	TJ208	59.8	19.6	0.956	0.33
	M. pudica inoculated with TJ208	32.6	42.9	0.982	1.32
	M. pudica without inoculation	25.5	25.3	0.927	0.99

[a] *M. pudica* seedlings with or without inoculation after 3 weeks.
[b] $\eta = q_{max}/K_d$.



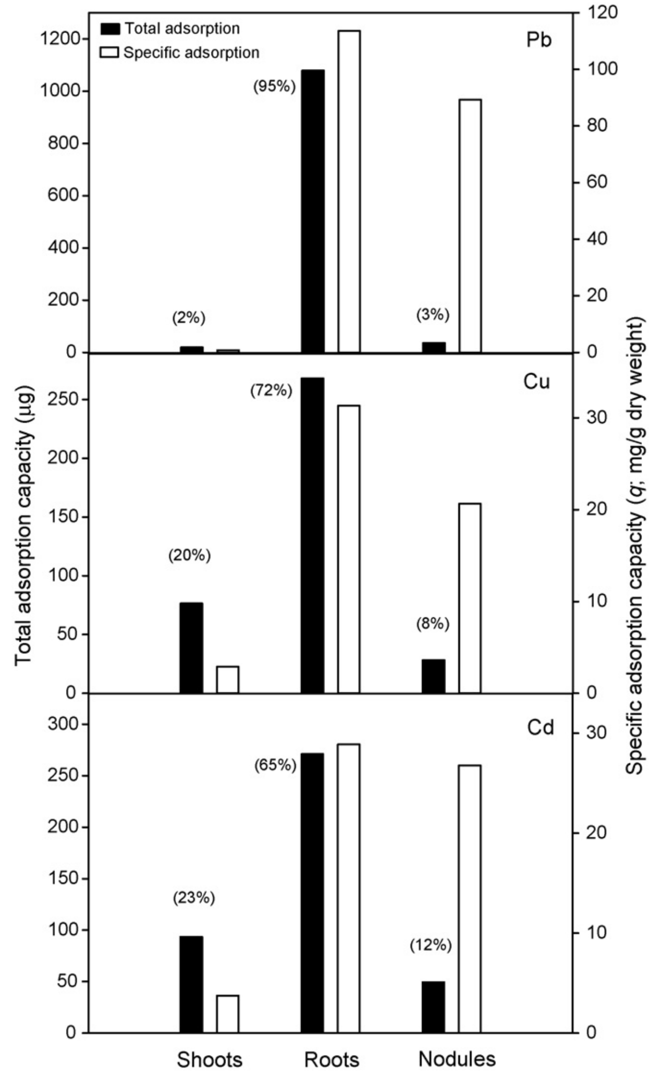


Fig. 4. Total and specific adsorption capacity of Pb, Cu, and Cd by *M. pudica* containing nodules of *C. taiwanensis* TJ208 (solid bars: total capacity; open bars: specific capacity; values in parentheses: percentages out of total adsorption capacity).

This demonstrates the feasibility and effectiveness of using this rhizobial-plant symbiotic system for the clean-up of heavy metals. The metal adsorption capacity in different parts of the plants was compared for 3-week-old nodulated plants exposed to 100 mg l^{-1} of Pb, Cu, and Cd. The roots contributed to the majority of total metal uptake (especially for Pb adsorption), followed by shoots and then the nodules (Fig. 4). However, if the specific adsorption capacity (q; the adsorption capacity per unit dry weight of biomass) is compared this shows that the roots actually had the highest q values of 113.6, 31.3, and 28.9 mg (g dry weight)$^{-1}$ for Pb, Cu, Cd, respectively, with values for nodules being only slightly less at 89.3, 20.7, and 26.8 mg (g dry weight)$^{-1}$ for Pb, Cu, Cd, respectively (Fig. 4). In contrast to

Fig. 3. Adsorption isotherms of (a) Pb, (b) Cu and (c) Cd by *Mimosa pudica* alone, *C. taiwanensis* TJ208 alone, and a symbiotic system of *M. pudica* with TJ208 nodules (symbols: experimental data; curves: simulation with Langmuir isotherm).

the roots and nodules, the shoots had negligible q values (less than 3.8 mg/g dry weight).

4. Discussion

4.1. Significance of the C. taiwanensis isolates in environmental applications

The genus *Cupriavidus* (formerly named *Ralstonia*) is known to possess the ability of biodegradation and resistance to organic (e.g., phenolics or chlorinated aromatic/aliphatic compounds) and inorganic pollutants (e.g., heavy metals), and hence it is of great interest in the field of environmental biotechnology. Recent work has shown that a *C. taiwanensis* isolate (strain TJ186) was able to degrade both phenol and trichloroethylene (TCE) efficiently [30,36]. In this study, another *C. taiwanensis* strain, TJ208, has been shown to have high resistance to a variety of heavy metals and is also capable of adsorbing heavy metals (Table 2 and Fig. 2). Compared to a well-known metal-resistant strain, *C. metallidurans* CH34 [31], strain TJ208 had similar MIC values with a similar trend of metal dependence (Table 1), thus suggesting that they are comparable in terms of metal resistance. This heavy-metal resistance combined with their ability to cope with organic and inorganic pollutants [30,36], shows that some *C. taiwanensis* strains may be well suited for cleaning up environments contaminated by multiple types of pollutants.

4.2. Performance of the C. taiwanensis isolate as a metal biosorbent

This study demonstrates that *C. taiwanensis* TJ208 possesses the ability to adsorb Pb, Cu, and Cd (Figs. 1 and 2). In terms of the weight-based maximum adsorption capacity (q_{max}), strain TJ208 had a particularly high capacity for Pb adsorption, while it's capacity for Cu and Cd adsorption was less than that for Pb, but these were similar to each other. The same trend was observed for adsorption rates (r_{ads}), as the ranking for r_{ads} was Pb > Cu > Cd. Note that the information regarding the rate of biosorption is rarely discussed in the literature, but the rate values are provided in this work to represent the key parameter of biosorption kinetics. Moreover, since the adsorption efficiency is positively proportional to q_{max} and inversely proportional to K_d, the overall adsorption efficiency (η) can be expressed by the ratio of the two parameters (i.e., $\eta = q_{max}/K_d$). Table 2 shows that the η value decreased in the order of Pb (1.06) > Cu (0.69) > Cd (0.33) (Table 2). Therefore, based upon both kinetic and thermodynamic characteristics of biosorption, strain TJ208 appeared to exhibit significantly higher biosorption performance for Pb, while Cd adsorption was the least efficient for the three metal adsorbates tested. This trend is consistent with the findings of previous reports using different bacterial biosorbents [32,37–39]. Although it was found that *C. taiwanensis* TJ208 as a metal biosorbent had comparable metal adsorption capacities to the reported values [2–4], as indicated in Fig. 3 and Table 2, a more important role of strain TJ208 strain was to trigger a drastic increase in the metal biosorption performance of plants in a symbiotic system with strain TJ208 housed in root nodules on the host plant (*M. pudica*). This symbiotic system could be used for phytoremediation of metal-contaminated soil or underground water via batch or continuous operation mode.

4.3. Promoting metal uptake with a symbiotic system of C. taiwanensis and M. pudica

Interestingly, although free-living strain TJ208 had a lower capacity for metal adsorption than non-nodulated *M. pudica*, nodulation by this rhizobial strain appeared to promote metal adsorption by the plants to a significant extent (Fig. 3). The nodulated plants had 12–86% higher metal uptake capacity (in terms of q_{max}) than the nodule-free plants. Also, the adsorption efficiency (η) of nodulated plants was much higher than the nodule-free ones, resulting in a 71, 81, and 33% enhancement in the adsorption efficiency of Pb, Cu, and Cd, respectively (Table 2). It is also noted that nodulation had the greatest enhancing effect on Pb uptake. This is consistent with the fact that strain TJ208 had the highest ability for Pb biosorption (Fig. 2 and Table 2), reflecting the major role that the rhizobial strain could play in the enhancement of metal uptake via formation of root nodules. Therefore, the stimulating effect of nodulation on metal uptake ability may be two-fold:

1. The root nodules contained high concentrations of rhizobia (TJ208), thereby acting as metal biosorbents and contributing to part of metal uptake. The specific metal adsorption capacity of nodules was comparable to that of the plant roots, indicating the important role that nodules played in metal uptake by the plants (Fig. 4).
2. The nodules served as metal buffer zones, providing extra protection for the plant against invading metal ions and other soluble contaminants, since the contaminants (including metal ions) would inevitably make contact with the nodules. This also forms a multi-stage metal biosorption process, as roots and nodules adsorb metal ions in the first stage, followed by metal uptake by roots and nodules, and finally by shoots. This multi-stage metal uptake somehow seemed to be beneficial to overall metal uptake capacity and efficiency (Fig. 3 and Table 2) and may also protect the plants from direct exposure to adverse environments (e.g., high heavy-metal concentration).

4.4. Distribution of metal accumulation in nodulated plants

Using inactivated or living plants as biosorbents for metal removal has gained increasing interest amongst researchers for the past decades [2–4]. However, there is little information regarding the use of nodulation as a strategy to stimulate metal adsorption capacity and efficiency of the plants. It is known that plants possess cysteine-rich metal-binding proteins, called phytochelatins [40], which contribute significantly to the metal uptake in plants. These proteins are mainly present in plant roots [40], which is consistent with the observations in the present study that the roots accumulated the majority of invading metal ions, accounting for 95, 72, and 65% of the total uptake of Pb, Cu, and Cd, respectively, in a nodulated plant (Fig. 4). On the other hand, the root nodules containing a large amount of strain TJ208, thereby also contributing to a proportion (3–12%) of metal uptake. It is surprising to discover that the nodules had a comparable specific adsorption capacity (q) to the roots (Fig. 4) even though the plants in general had higher metal uptake capacity than strain TJ208 itself (Fig. 3). This implies that TJ208 nodules had much higher uptake capacity than the suspended free-living TJ208 cells. In other words, nodulation by TJ208 seemed to markedly increase its metal uptake capacity. This could be due to certain physiological changes through the formation of TJ208 bacteriod cells within nodules, leading to the increase in metal-binding sites. Or, it could be due to the release of some extracellular products (e.g., extracellular polysaccharides) during formation of the nodules [41,42], creating a large number of additional metal-binding sites. Rhizobial bacteroids in the nodules might also help to increase the uptake of heavy metals via plant roots by altering mobility of metals. This symbiotic association might have the potential to enhance root absorption, and stimulate the acquisition of plant nutrients, including metal ions. In contrast to the roots, the shoots, despite containing over 80% of the total plant biomass, made only a minor contribution to the overall metal uptake capacity, accounting for only 2, 20, and 23% of total adsorption capacity for Pb, Cu, and Cd, respectively. The low capacity for shoots may be attributed to the lack of metal-binding proteins or relevant substances in their constituents. Also, as mentioned earlier, the metal uptake by nodulated *M. pudica* was essentially a multi-stage process, in which metal sorption by stems and leaves was the final stage. Thus, a large proportion of invading metals could be taken up by roots and root nodules so that the shoots had less exposure to the metals, hence resulting in a lower contribution to total metal uptake. Accumulation of metals in the roots (and root nodules) may be less harmful to the plants while being exposed to the metal-polluted environment, resulting in better metal tolerance and plant survival. It is also desirable for crops and fruit plants to accumulate most metal contaminants in the roots so that the grains and fruits will be less contaminated. Therefore, the concept of using nodulated plants to immobilize most environmental heavy-metal contaminants in the roots and root nodules could also be applied to other (nodulated) plants for either directly enhancing their metal tolerance or by restricting the migration of metal contaminants to less harmful and more controllable regions within the plants.

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