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Cr(VI) Resistance and Removal by Indigenous Bacteria Isolated from Chromium-Contaminated Soil

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The removal of toxic Cr(VI) by microorganisms is a promising approach for Cr(VI) pollution remediation. In the present study, four indigenous bacteria, named LY1, LY2, LY6, and LY7, were isolated from Cr(VI)-contaminated soil. Among the four Cr(VI)-resistant isolates, strain LY6 displayed the highest Cr(VI)-removing ability, with 100 mg/l Cr(VI) being completely removed within 144 h. It could effectively remove Cr(VI) over a wide pH range from 5.5 to 9.5, with the optimal pH of 8.5. The amount of Cr(VI) removed increased with initial Cr(VI) concentration. Data from the time-course analysis of Cr(VI) removal by strain LY6 followed first-order kinetics. Based on the 16S rRNA gene sequence, strain LY6 was identified as *Pseudochrobactrum asaccharolyticum*, a species that had never been reported for Cr(VI) removal before. Transmission electron microscopy and energy dispersive X-ray spectroscopy analysis further confirmed that strain LY6 could accumulate chromium within the cell while conducting Cr(VI) removal. The results suggested that the indigenous bacterial strain LY6 would be a new candidate for potential application in Cr(VI) pollution bioremediation.

Keywords: Bioremediation, Cr(VI) resistance, Cr(VI) removal, kinetics, *Pseudochrobactrum asaccharolyticum*

Introduction

As an important industrial material, chromium is widely and extensively used in pigment manufacturing, electroplating, leather tanning, wood preservation, and alloy production [38, 42, 55]. Chromium exists in a wide range of valence states, with hexavalent species [Cr(VI)] being considered as the most toxic form of chromium [13]. Cr(VI) is highly soluble, oxidizing, mutagenic, and carcinogenic [1, 17]. Therefore, the inappropriate disposal of Cr(VI)-containing byproducts and uncontrolled release of Cr(VI) wastes have caused serious environmental pollution problems [9]. Conventional technologies for Cr(VI) contaminants treatment, including chemical reduction, precipitation, ion exchange, and reverse osmosis, cannot be applied in large scale because of the high cost and subsequent secondary environmental pollution [3, 4, 37]. Alternatively, bioremoval of Cr(VI) by microorganisms has been considered as a promising process for treating Cr(VI)-contaminated wastes owing to its cost-effective and eco-friendly advantages [6, 7, 33].

Resistance and removal of Cr(VI) have been reported in a variety of aerobic, facultative, and anaerobic bacterial strains, such as *Vibrio fischeri* [18], *Aspergillus niger* [52], *Shewanella alga* [20, 21], *Paracoccus denitrificans* [29], *Pseudomonas* sp. [30], *Bacillus subtilis* [34], *Bacillus* sp. [28, 36], and *Shewanella oneidensis* [35]. However, most studies have shown that these isolates are capable of removing Cr(VI) at low concentrations, usually no more than 0.5 mM (26 mg/l) [8, 24, 44, 46, 56]. Some microorganisms even lose Cr(VI)-removing ability in the presence of high Cr(VI) concentrations, which further complicates the bioremediation of Cr(VI)-contaminated soils [32, 38]. In addition, most of these microorganisms were isolated from polluted waters or sewages. The use of these exogenous strains for soil bioremediation might lead to soil ecology risk because of

the competition between exogenous strains and indigenous microorganisms [7]. Thus, to develop eco-friendly and cost-effective techniques for removing Cr(VI) pollutants from contaminated soil, indigenous microorganisms capable of tolerating and removing high Cr(VI) concentrations need to be isolated and further characterized [8, 32].

The objective of the present study was to isolate promising indigenous bacteria from Cr(VI)-contaminated soil and to describe the characteristics of their Cr(VI) resistance and removal abilities. The superior Cr(VI)-removing competence of a new *Pseudochrobactrum asaccharolyticum* strain was further focused upon and intensively investigated.

Materials and Methods

Inoculum Source and Growth Medium

Surface soil samples (0−15 cm depth) were collected aseptically in sterile flasks from a long-term chromium slag-contaminated site in Hangzhou, Zhejiang Province (30°15′N; 120°10′E), in the southeast of China. The samples were transported to the laboratory in ice [40], and used as inoculum source to obtain indigenous isolates capable of removing Cr(VI). The total concentrations of Cr, Pb, Zn, Cd, and Cu in this sample were determined by atomic absorption spectrometry [22]. The water-soluble Cr(VI) was analyzed by using the diphenylcarbazide (DPC) method [53]. Specifically, water-soluble Cr(VI) was initially extracted with distilled water at a ratio of 1:10 (w/v) for 8 h. Then the soil-water mixture was centrifuged at 13,800 ×g for 30 min. One milliliter of the supernatant was drawn and added into a 50 ml measuring flask. After being mixed with 0.5 ml of H_2SO_4 (0.5 M), 0.5 ml of H_3PO_4 (0.5 M), and 2 ml of DPC (0.2% in acetone), the solution was measured at a wavelength of 540 nm by a spectrophotometer.

Modified Luria-Bertani (LB) medium used for microorganism enrichment and culture consisted of 10 g tryptone, 5 g NaCl, and 5 g yeast extract in 1,000 ml of distilled water [5, 50]. The pH was adjusted to 7.2 by using either HCl or NaOH. All media were autoclaved at 121°C for 30 min prior to use. In all the following experiments, a filter-sterilized solution of potassium dichromate was used as the source of Cr(VI) [50].

Isolation of Cr(VI)-Resistant Bacterial Strains

Enrichment of Cr(VI)-removing microorganisms was carried out immediately after transporting the samples into the laboratory. Two grams of soil sample was transferred into 50 ml of LB medium and incubated at 28°C for 24 h with agitation (180 rpm). Thereafter, the culture medium was decanted and diluted into a series of dilution sequences. One tenth milliliter of 10^{-6}, 10^{-7}, and 10^{-8} dilution series were separately spread on LB agar plates containing 260 mg/l Cr(VI). After incubation at 28°C for 4 days, four bacterial colonies of different morphology were selected for further studies and named LY1, LY2, LY6, and LY7, respectively.

Cr(VI) Resistance and Removal by Different Isolates

The Cr(VI) resistance of different isolates was evaluated using agar and shake-flask techniques modified from that described by Pei *et al.* [39].

Isolated colonies were picked up with a sterilized wire loop and streaked on LB agar plates supplied with a series of Cr(VI) concentrations (from 52 to 520 mg/l). The cultures were incubated at 28°C for 48 h. Cr(VI) resistance was measured as the number of colonies formed after the incubation period.

For the shake-flask technique, overnight cultures of four different isolates (1% (v/v)) were inoculated respectively into 50 ml of fresh LB liquid medium added with 260 mg/l Cr(VI). The samples were incubated at 28°C, with 180 rpm shaking for 84 h. Three milliliter aliquots were sampled at regular time intervals and analyzed for growth at OD 600 nm.

At the end of the incubation, aliquots of solution were taken out using a syringe and centrifuged at 13,800 ×g, 4°C for 10 min. The supernatants were analyzed for residual Cr(VI) concentration. A similar experimental setup without bacterial cells served as a control, and the experiments were performed with three replicates.

Time-Course Study on Bacterial Growth and Cr(VI) Removal by Strain LY6

Out of the four isolates, strain LY6 showed the highest Cr(VI)-removing potential. Thus, its growth and Cr(VI)-removing course were further analyzed.

A purified colony of the strain was inoculated into 100 ml of liquid LB medium and incubated in a shaker with a speed of 180 rpm at 28°C overnight (16−18 h). Thereafter, 500 μl of the grown bacterial culture was transferred into 50 ml of fresh liquid LB medium containing 300 mg/l Cr(VI), and incubated at 28°C by shaking at a speed of 180 rpm for 60 h. Aliquots of solution were withdrawn using a syringe at 0, 12, 24, 36, 48, and 60 h. Samples were then divided into two portions. One portion was centrifuged and supernatants were analyzed for residual Cr(VI) concentration. The other portion was used to evaluate cell growth by measuring the optical density at 600 nm [31]. A control treatment without bacterial strain inoculation was incubated in parallel. All the experiments were performed with three replicates.

Effects of pH and Cr(VI) Concentration on Cr(VI) Removal by Strain LY6

The effects of initial pH and Cr(VI) concentration on Cr(VI) removal by LY6 were characterized at the same growth condition as the previous incubations (28°C, 180 rpm). For determination of the optimum pH, liquid LB media containing 104 mg/l Cr(VI) were prepared in 7 sets and their pH were adjusted to 5.5, 6.5, 7.5, 8.5, 9.5, 10.5, and 11.5, separately. After an incubation of 60 h, aliquots of different solutions were withdrawn and residual Cr(VI) concentrations were analyzed as described above.

Bioremediation of Cr contamination has been regarded as a promising and cost-effective technology, especially when dealing

with Cr(VI)-containing wastes at low-to-mid concentrations (10–200 mg/l) [12]. Thus, from the application point of view, initial Cr(VI) concentrations ranging from 10 to 200 mg/l (10, 20, 50, 100, 150, and 200 mg/l, respectively) were used for simulating the water-soluble Cr(VI) in the polluted soil. At regular intervals, 1 ml of different cultures were separately taken out in sterilized tubes, and the aqueous Cr(VI) concentrations were determined.

Identification of Cr(VI)-Removing Strain

Genomic DNA of strain LY6 was extracted using the UNIQ-10 Column Bacterial Genomic DNA Isolation Kit (Sangon Biotech Co., Ltd., China). Bacterial 16S rRNA gene primers 1541R (5′ AAG GAG GTG ATC CAG CCG CA 3′) and 8F (5′ AGA GTT TGA TCC TGG CTC AG 3′) were used for polymerase chain reaction (PCR) amplification. The PCR amplification was performed as follows: each reaction was performed in a final volume of 50 μl, containing 0.25 μl Taq polymerase, 5 μl of 10×PCR Buffer, 3 μl of MgCl$_2$, 4 μl of dNTP mixture, 1 μl of each primer, 2.5 μl of DNA sample (2.5 ng), and 33.25 μl of deionized water. The reaction mixture was subjected to 30 cycles of amplification as follows: initial denaturation at 94°C for 3 min; subsequent denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min 30 sec. The amplified products were analyzed by electrophoresis on 1.2% agarose–TBE gels, stained with ethidium bromide (0.5 μg/ml) and visualized on a UV transilluminator. The PCR products were purified using the UNIQ-10 Spin Column PCR Product Purification Kit (Sangon Biotech Co., Ltd.) and sent to Biological Company (China) for sequencing [7]. The sequences were initially analyzed in the NCBI server (http://www.ncbi.nlm.nih.gov/) using BLAST and corresponding sequences were downloaded [50]. The bacteria nucleotide sequences were aligned by using the CLUSTALX program. A phylogenetic tree was constructed by the neighbor-joining method using the Paup program.

TEM-EDS Analysis

Cells of strain LY6 grown in liquid LB medium containing 156 mg/l Cr(VI) for 48 h were centrifuged at 12,000 ×g, 4°C for 10 min. Pellets obtained were prepared for TEM examination using the method of Chen et al. [10]. The sample was fixed in 2.5% glutaraldehyde at 4°C overnight. Fixed cells were rinsed three times with 0.1 mM phosphate buffer (pH 7.0) and post-fixed for ~1–2 h in 1% osmic acid. The pellets were subsequently dehydrated with different concentrations of ethanol (50%, 70%, 80%, 90%, and 95%) for 15 min each. After dehydration in the 100% ethanol and acetone series for 20 min, the cell pellets were then saturated in 50% and 75% (v/v) of epoxy resin in acetone for 1 and 3 h,

respectively. Samples were stored in pure epoxy resin overnight at 70°C. Ultrathin sections (~70–90 nm) were cut on a Reichert Microtome (Reichert-Jung, Austria), and stained with uranyl acetate and lead citrate for 15 min each. The specimens were photographed using a transmission electron microscope (Hitachi-7650, Japan). Energy-dispersive spectroscopy of chemical elements in bacteria was performed with an INCA EDS system (Oxford-INCA).

Cr(VI) Analytical Method

The concentration of hexavalent chromium was determined colorimetrically at 540 nm using the DPC method with a detection limit of 0.2 μg/l [53]. In a 50 ml volumetric flask, 1 ml of sample was mixed with 0.5 ml of 0.5 M H$_2$SO$_4$ and 0.5 ml of 0.5 M H$_3$PO$_4$. Then 2 ml of freshly prepared 0.2% (w/v) DPC in acetone was added to the volumetric flask. The mixture was measured at OD$_{540}$ and distilled water was used as a reference.

Statistical Analysis

Analysis of variance was performed using SPSS 13.0 (SPSS Inc., Chicago, USA). Effects of different treatments were compared by one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered to be statistically significant [49].

A first-order model was used to determine microbial Cr(VI) removal kinetics in the presence of different initial Cr(VI) concentrations [15]:

$$Cr(VI) = Cr(VI)_o e^{-kt}$$

where Cr(VI) is the model predicted concentration of chromium (mg/l), Cr(VI)$_o$ is the initial Cr(VI) concentration (mg/l), k is the first-order rate constant, and t is the time (h).

Results and Discussion

Isolation of Cr(VI)-Resistant Bacterial Strains

The soil chosen for Cr(VI) resistance bacteria screening was classified as sandy soil according to the FAO/UNESCO soil classification system [7]. The physical and chemical properties are presented in Table 1. The soil was seriously polluted by hexavalent chromium and other heavy metals as well. The water-soluble Cr(VI) of the soil was 244.7 mg/kg, and the total Cr was as high as more than 20,000 mg/kg. The total concentrations of Pb, Zn, Cd, and Cu were 287.4, 575.1, 8.6, and 17.3 mg/kg, respectively.

Heavy-metal contamination is known to cause shifts in microbial communities with the emergence of bacterial

Table 1. Physical and chemical properties of the sample soil.

Soil type	pH	Organic matter (%)	Pollutants concentrations (mg/kg)					
			Total Cr	Water-soluble Cr(VI)	Pb	Zn	Cd	Cu
Sandy soil	8.75 ± 0.1	0.13 ± 0.02	20,392.0 ± 32.6	244.7 ± 6.5	287.4 ± 3.1	575.1 ± 10.6	8.6 ± 0.9	17.3 ± 1.2

species with elevated metal resistance [14, 47]. Therefore, the severe chromium contamination served as a selective pressure on the microbial flora of polluted soils [54]. After enrichment and isolation, four morphologically different isolates were able to grow on plates amended with 260 mg/l Cr(VI), and named LY1, LY2, LY6, and LY7, respectively.

Cr(VI) Resistance and Removal by Different Isolates
Cr(VI) resistance of four isolates in different medium.
The minimum inhibitory concentration (MIC) of Cr(VI) on LB agar to the bacterial isolates is the lowest concentration at which a single colony-derived streak could not grow [41]. According to the Cr(VI) resistance experiments, the MICs to LY1, LY2, LY6, and LY7 were 416, 416, 364, and 312 mg/l Cr(VI), respectively, with few colonies observed (<10). However, the growth responses of the four strains toward 260 mg/l Cr(VI) in LB liquid media were quite different. As shown in Fig. 1, strains LY6 and LY7 grew very well at the concentration of 260 mg/l Cr(VI). There was no remarkable difference in curve patterns of both strains among the tested concentration, indicating that the strains LY6 and LY7 were tolerant to Cr(VI) concentrations as high as 260 mg/l in liquid media. However, in the liquid media containing 260 mg/l Cr(VI), the growth of strains LY1 and LY2 was significantly hindered, although both of them could tolerate 416 mg/l Cr(VI) on LB plates. The maximum biomass of strain LY1 was much less than those of strains LY6 and LY7. Meanwhile, the growth of isolate LY2 was greatly inhibited, and no obvious biomass increase was observed. The results demonstrated that the

Cr(VI) resistance of four strains was quite different in liquid media as compared with that on agar plate, and some of them (LY1 and LY2) showed lower tolerance in liquid media than on agar plate. Our results were in agreement with the study of Megharaj *et al.* [31]. Although *Bacillus* sp. and *Arthrobacter* sp. exhibited similar resistance on agar media supplemented with 100 mg/l Cr(VI), *Bacillus* sp. could not grow in liquid medium containing 100 mg/l Cr(VI).

The results indicated that the incubation conditions were of vital importance in determining the chromium resistance of bacteria. It also suggested that the actual resistance of the bacteria to a pollutant should be determined preferably in liquid media after their isolation from agar plates [31]. Metals were relatively more freely available in liquid than in solid medium, thereby increasing the metal toxicity in liquid culture [43]. Nevertheless, the test of toxicity in solid media investigated here could be useful in the evaluation of chromium toxicity in contaminated soil and sewage sludge, where conditions of complexation and availability of metals were different from those observed in liquid media [11].

Cr(VI) removal by different isolates. An interesting resistance-removal contradiction was observed while the Cr(VI)-removing abilities of different isolates were being detected. Among four bacterial isolates, strain LY6 demonstrated the highest Cr(VI)-removing ability, with more than 80 mg/l Cr(VI) being removed within 84 h. However, strains LY1 and LY2, both of which showed the same highest tolerance on LB plate (416 mg/l Cr(VI)), removed only 18.9 and 29.8 mg/l Cr(VI), respectively. This resistance-removal inconsistency was also observed in strains LY6 and LY7. Even though there was no remarkable difference in their growth curve in liquid media added with Cr(VI), their Cr(VI) removal abilities varied from each other. LY6 reduced much more Cr(VI) than that removed by LY7 at the same period.

The results indicated that the Cr(VI)-removing capability of the microbial strain was not related to Cr(VI) resistance ability and was growth independent (Figs. 1 and 2). Our results were in agreement with the work reported by Megharaj *et al.* [31]. No relation was found between the growth of *Arthrobacter* sp. and its removal of Cr(VI). Similarly, Chai *et al.* [7] found that the final biomass levels of bacteria BB, BY, and BW did not effect their Cr(VI) removal capacities. These further implied that the isolates with low chromate resistance would also be able conduct effective Cr(VI) removal. This may be related to the activity of chromate reductases [51].
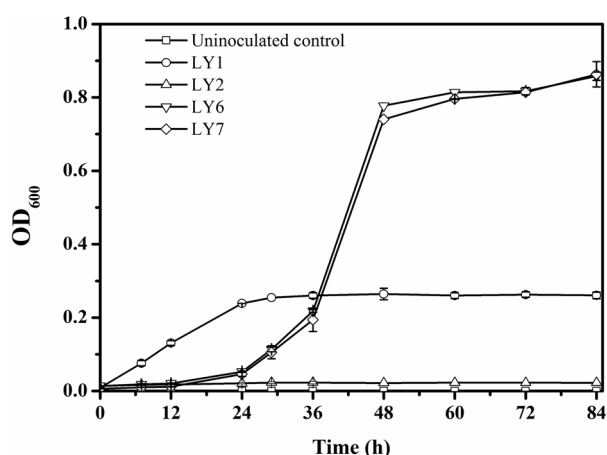

Fig. 1. Growth of different strains in the presence of 260 mg/l Cr(VI).
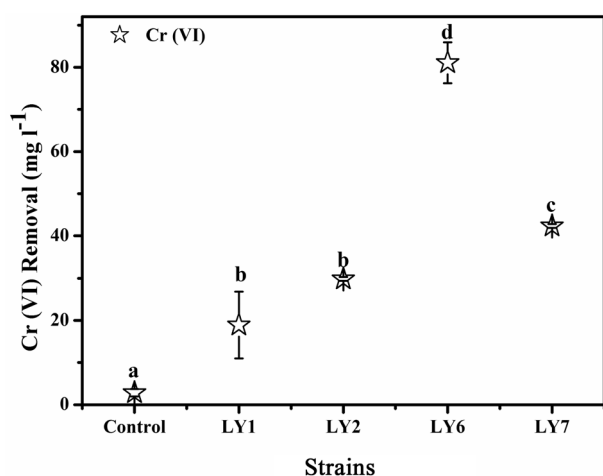Error bars represent the standard deviation of triplicate samples.

Fig. 2. Cr(VI) removed by different isolates after incubation for 84 h in liquid LB media containing 260 mg/l Cr(VI).
Treatments followed by the same letter are not significantly different at $p = 0.05$.

Time-Course Study on Bacterial Growth and Cr(VI) Removal by Strain LY6

The time course of Cr(VI) removal during the growth of LY6 is shown in Fig. 3. There was an obvious lag phase of 24 h at the high concentration of 300 mg/l Cr(VI). The reduced growth was likely related to the altered metabolic and physiological reactions of bacteria due to the high toxicity of Cr(VI) [26]. However, although the incipient bacterial growth was slow, nearly 40 mg/l of Cr(VI) decrease was obtained during the first 12 h, and the average removal rate during this period was as high as 3.3 mg l^{-1} h^{-1}. It was likely that some adsorption of Cr(VI) onto the bacterial cells contributed to the Cr(VI) decrease in the initial phase.
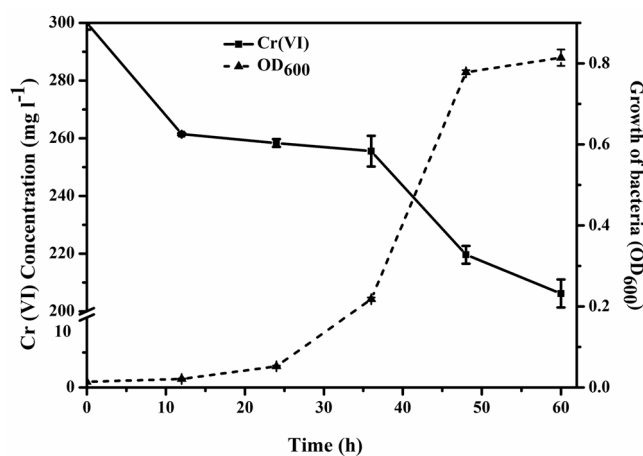

Fig. 3. Cell growth (triangle marker) and Cr(VI) reduction (square marker) of strain LY6.

After a short period of adjustment (24 h), strain LY6 exhibited a fast growth together with a strong Cr(VI)-removing ability. As the incubation time extended to 48 h, Cr(VI) was removed from the initial 300 mg/l to 220 mg/l. The biomass reached the maximum of cell growth at 60 h, while the removal rate was still maintained at a high level. During the sampling period, 94 mg/l Cr(VI) was removed with an average removal rate of 1.6 mg l^{-1} h^{-1}.

In previous literature, the Cr(VI) removal rates and removing abilities of most reported isolates were relatively low. *Bacillus* sp. RE could remove 50% of 80 mg/l Cr(VI) after 42 h. However, its Cr(VI)-removing efficiency decreased with increasing Cr(VI) concentration [16]. In the study by McLean and Beveridge [30], pseudomonad strain CRB5 took 120 h to completely remove 20 mg/l Cr(VI), with an average removal rate of 0.17 mg l^{-1} h^{-1}. *D. acidovorans* AR merely removed approximately 60% Cr(VI) after 10 h growth at a very low initial concentration of 2.5 mg/l Cr(VI) [19]. Bacterial cell of *Pseudomonas* sp. even could not survive for long at the concentration of 4 mg/l Cr(VI) [45]. However, in this study, strain LY6 was tolerant to 364 mg/l Cr(VI) and could remove about 94 mg/l Cr(VI) within 60 h. This observation was of significance in pursuing a new efficient Cr(VI) reducer, because strain LY6 demonstrated a much higher removal rate than many strains previously reported and proved to be a more competent candidate for Cr(VI) removal.

Effects of pH and Cr(VI) Concentration on Cr(VI) Removal by Strain LY6

Chromate removal by LY6 was found to be influenced by pH. As shown in Fig. 4, strain LY6 could effectively remove Cr(VI) at a wide pH range from 5.5 to 9.5. At the optimum pH of 8.5, more than 80% Cr(VI) removal was achieved within 60 h. However, strong alkaline pH (>10.5) distinctly decreased the Cr(VI)-removing ability and only 9.6% Cr(VI) was removed. Contrary results were reported by Elangovan *et al.* [16], where the chromate reductase activity of *Bacillus* sp. strain RE was observed in a narrow pH range with an optimum at 6.0. Acidic pH drastically decreased the enzyme stability, whereas alkaline pH slightly reduced the stability with retention of around 80% residual activity. The reason for the preference to low alkalescency was probably associated with the adaptation to the indigenous weak alkaline soil, from which strain LY6 was isolated (Table 1).

The effect of initial Cr(VI) concentration on Cr(VI)-removing ability was probed over a Cr(VI) range from 10 to 200 mg/l. A superior Cr(VI)-removing ability was observed
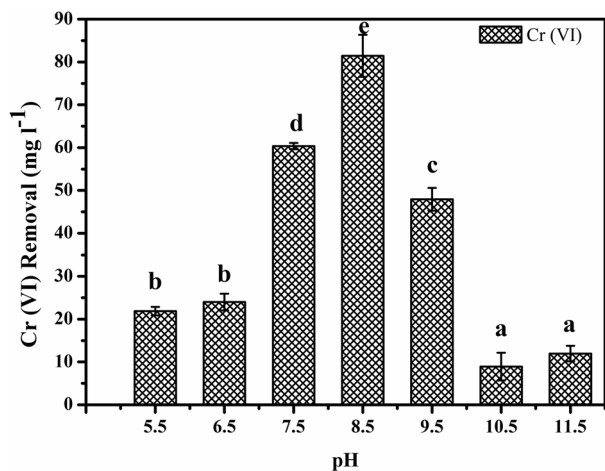
Fig. 4. Effect of pH on the Cr(VI) removal ability of strain LY6. Experiments were conducted with an initial concentration of 104 mg/l Cr(VI) and reduction time of 60 h. Treatments followed by the same letter are not significantly different at $p = 0.05$.

for strain LY6 (Fig. 5). It could completely remove 10, 50, and 100 mg/l Cr(VI) within 24, 48, and 144 h, respectively. Ninety-eight percent of 150 mg/l Cr(VI) was removed within 240 h. The time required for complete Cr(VI) removal extended with the initial Cr(VI) concentration. Further kinetic analysis indicated that data from the time course of Cr(VI) removal had a good fit to a first-order kinetic model ($R^2 = 0.969$-0.999) (Table 2). As seen from the results, the removal rate constant (k) decreased from 0.125 h^{-1} at 10 mg/l Cr(VI) to 0.007 h^{-1} at 200 mg/l Cr(VI). The reduced rate constant was probably related to the inhibitory effect of
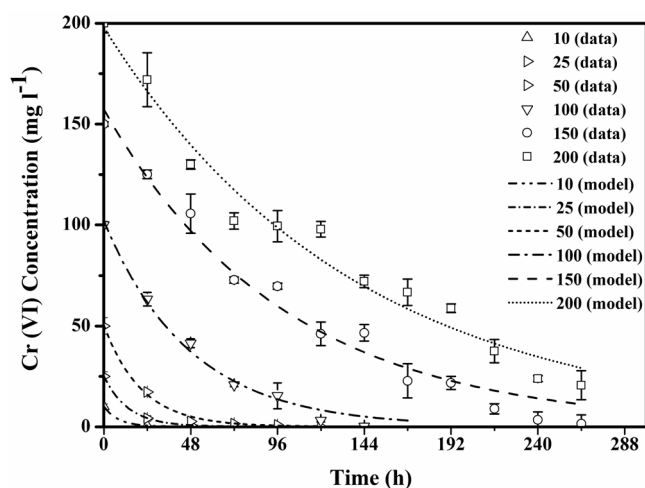
Table 2. First-order kinetic model equations and kinetic parameters for the removal of different concentrations of Cr(VI) by strain LY6.

Initial Cr(VI) concentrations (mg/l)	Rate constants (k) (h^{-1})	Cr(VI)= Cr(VI)$_o$e^{-kt}	R^2
10	0.125	Cr(VI)= 10e$^{-0.125t}$	0.999
25	0.074	Cr(VI)= 25e$^{-0.074t}$	0.999
50	0.048	Cr(VI)= 50.2e$^{-0.048t}$	0.994
100	0.021	Cr(VI)= 101.8e$^{-0.021t}$	0.988
150	0.010	Cr(VI)= 157.1e$^{-0.01t}$	0.973
200	0.007	Cr(VI)= 197.7e$^{-0.007t}$	0.969

Cr(VI) to the microorganism at higher concentrations [26]. However, although the removal rate constant decreased with the increase of initial Cr(VI) concentration, strain LY6 could still maintain effective Cr(VI)-removing activity at a concentration up to 200 mg/l Cr(VI). In addition, the amount of removed Cr(VI) increased with increasing concentration of Cr(VI). The result was consistent with the study of Megharaj *et al.* [31], where the total Cr(VI) removal by *Bacillus* sp. increased with Cr(VI) concentration escalating from 5 to 100 mg/l.

Identification of Strain LY6

The bacterial strain LY6 grew into creamish, circular colonies with entire smooth margin. Further identification was carried out by using the 16S rRNA gene sequence. The sequence size was 1,408 bp. Compared with known sequences in the nucleotide databases, this strain showed 99% similarity with *Pseudochrobactrum asaccharolyticum* (AM180485). The phylogenetic tree is shown in Fig. 6. On the basis of 16S rRNA gene sequence analysis, strain LY6 was identified as *Pseudochrobactrum asaccharolyticum*. The 16S rRNA gene sequence of LY6 was submitted to the NCBI GenBank with the accession number of KC618329. Several Cr(VI)-removing bacteria were isolated, such as *Desulfuromonas acetoxidans* [48], *Pseudomonas aeruginosa.*, *Mc. capsulatus* [23], and *Acinetobacter haemolyticus* [2]. However, to our knowledge, there was no report on Cr(VI) removal by *Pseudochrobactrum asaccharolyticum*. This finding will provide a new resource for microbial bioremediation of Cr(VI)-contaminated environments.

TEM-EDS Analysis

TEM analysis was carried out to detect possible morphology change and potential Cr precipitation during the Cr(VI) removal process. As shown in Figs. 7A–7D,
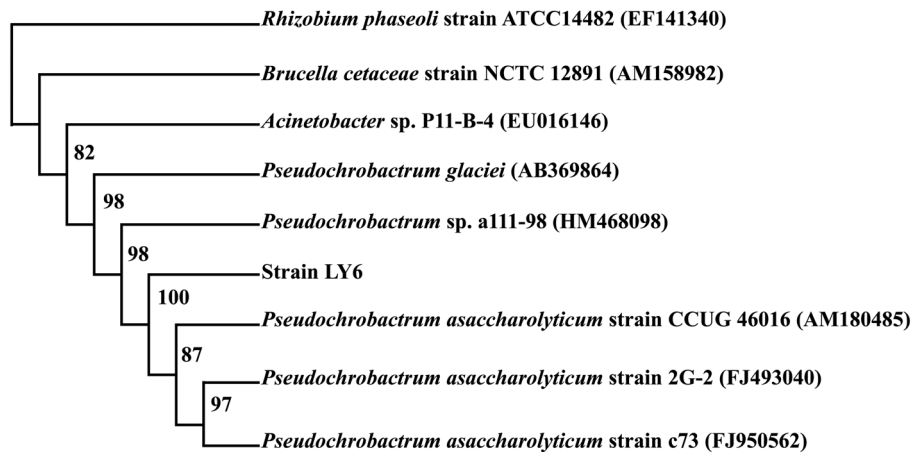

Fig. 5. Experimental and first-order model fitting results for Cr(VI) removal by strain LY6 at different initial Cr(VI) concentrations.

Fig. 6. Phylogenetic tree derived from 16S rRNA gene sequence data of strain LY6 and other related species.
The bar represents distance values; values at nodes represent percentage of 1,000 bootstrap replicates; the numbers in brackets represent GenBank accession numbers.

when LY6 was grown without Cr(VI) (Figs. 7A, 7B), the cells appeared as rods, and the inner and outer sections of bacteria could be clearly discerned. However, upon exposure to 156 mg/l Cr(VI) (Figs. 7C, 7D), the shape of cells became irregular, but no rupture of cells was detected.

Some strains were reported to experience marked change in size upon exposure to Cr(VI). It was stated that the cells of *P. putida* became both longer and wider after exposure to 780 mg/l Cr(VI), but reduced in size with 1,560 mg/l Cr(VI) exposure [27]. The increase in size was also observed for *A. haemolyticus* after exposure to 50 mg/l Cr(VI) [57]. However, no size change was detected for cells of strain LY6.

TEM analysis also revealed the presence of electron opaque particles, which were located mostly in the cytoplasmic region (Figs. 7C, 7D). This pattern was not found in thin sections of LY6 grown without Cr(VI) (Figs. 7A, 7B). Similar findings were also reported for *Ochrobactrum anthropi* [25] and *A. haemolyticus* [39]. EDS analysis further confirmed that the electron-opaque particle within the cell was a result of chromium precipitation (Fig. 7E). Since Cr(VI) is highly soluble, whereas Cr(III) displays a strong affinity for organics resulting in the formation of complex precipitation (*e.g.*, amorphous hydroxide) [13], it is tempting to speculate that the chromium in the cells of strain LY6 is in a reduced Cr(III) form resulting from the Cr(VI)-reducing process [7].

In the present study, the inconsistency of Cr(VI) resistance and removal ability was observed for four indigenous bacteria isolated from Cr(VI)-contaminated soil. A strain with high Cr(VI)-removal ability was identified as *Pseudochrobactrum asaccharolyticum*, a species that had been barely studied for

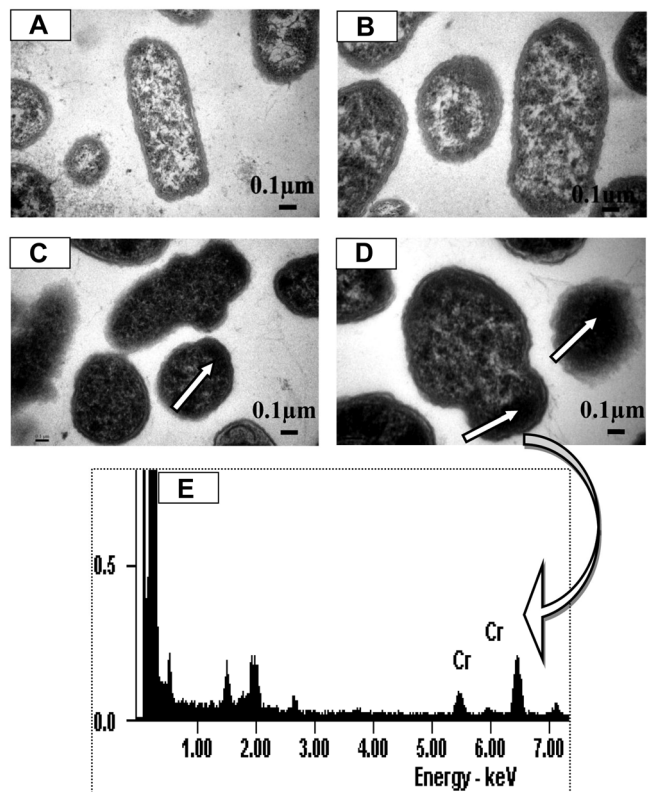Cr(VI) removal before. The *P. asaccharolyticum* strain LY6 demonstrated high Cr(VI) tolerance as well as superior

Fig. 7. TEM-EDS images of LY6 cells.
Cells grown without Cr(VI) (**A**, **B**), cells treated with 156 mg/l Cr(VI) (**C**, **D**), corresponding ED spectra of bacteria treated with 156 mg/l Cr(VI) (**E**). White arrows in C and D indicate electron-opaque particles.

Cr(VI) removal ability. The preference to neutral-alkalescency and efficient removal at high Cr(VI) concentration would facilitate its application in practical bioremediation. The ability to accumulate chromium within the cell was also observed for strain LY6. Results of this research would offer a potential candidate for Cr(VI) bioremediation, and the use of this indigenous bacterium might be helpful for the bioremoval of Cr(VI) in contaminated soils

Acknowledgments

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