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Cr (VI) remediation by indigenous bacteria in soils contaminated by chromium-containing slag

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ABSTRACT

Hexavalent chromium (Cr) is a toxic element causing serious environmental threat. Recently, more and more attention is paid to the bio-remediation of Cr (VI) in the contaminated soils. Cr (VI) remediation by indigenous bacteria in soils contaminated by chromium-containing slag at a steel-alloy factory in Hunan Province, China, was investigated in the present study. The results showed that when sufficient nutrients were amended into the contaminated soils, total Cr (VI) concentration declined from the initial value of 462.8 to 10 mg kg^{-1} at 10 days and the removal rate was 97.8%. Water soluble Cr (VI) decreased from the initial concentration of 383.8 to 1.7 mg kg^{-1}. Exchangeable Cr (VI) and carbonates-bound Cr (VI) were removed by 92.6% and 82.4%, respectively. Meanwhile, four Cr (VI) resistant bacterial strains were isolated from the soil under the chromium-containing slag. Only one strain showed a high ability for Cr (VI) reduction in liquid culture. This strain was identified as *Pannonibacter phragmitetus* sp. by gene sequencing of 16S rRNA. X-ray photoelectron spectroscope (XPS) analysis indicated that Cr (VI) was reduced into trivalent chromium. The results suggest that indigenous bacterial strains have potential application for Cr (VI) remediation in the soils contaminated by chromium-containing slag.

1. Introduction

Chromium (Cr (VI)) contamination in soils mainly results from the discharge of chromium-containing waste and waste-water from ore refining, production of steel and alloys, metal plating, tannery, wood preservation, and pigmentation. In soil environment, the most stable oxidation states of chromium are Cr (III) and Cr (VI). Cr (VI) is toxic and carcinogenic to humans via inhalation for long exposures [1,2]. Furthermore, Cr (VI) in soils can be leached into surface water or groundwater because of its high solubility and mobility. Concentrations of Cr (VI) as low as 0.5 mg L^{-1} in solution and 5 mg kg^{-1} in soils can be toxic to plants [3]. Hence, Cr (VI) is a significant risk to human health when it released into the soil environment.

Conventional technologies for Cr (VI) remediation in soils are physico-chemical extraction, land filling, stabilization/solidification, soil washing, flushing and excavation. However, most of these methods require high energy and large quantities of chemical re-agents [4], which could result in occurrence of secondary pollution. Thus, these technologies are not completely applied on a large scale. Recently, biological treatments arouse great interest in Cr (VI) remediation of contaminated sites because it is an economical and environmentally friendly way as compared to the conventional technologies. The bio-remediation strategy is to convert Cr (VI) into less toxic and less mobile Cr (III). Consequently, Cr (III) is immobilized in the soil matrix.

Many microbes were reported to reduce Cr (VI) under aerobic and anaerobic conditions [5–9]. Bio-reduction of Cr (VI) can be directly achieved as a result of microbial metabolism [10] or indirectly achieved by a bacterial metabolite such as H_2S [11–13]. Various bacteria were reported to remedy Cr (VI) contamination in soils. For instance, Jeyasingh and Philip [4] isolated a bacterial strain from a highly contaminated site and found the strain could reduce 5.6 mg g^{-1} Cr (VI) within 20 days in soil reactors. Desjardin et al. [14] found that Cr (VI) in soils was reduced by *Streptomyces thermocarboxydus* isolated from the contaminated soil. Bader et al. [15] studied Cr (VI) reduction in soil by microbial community under aerobic conditions and found that Cr (VI) was reduced by as much as 33% within 21 days. Virtually, most of the previous researches on biological reduction of Cr (VI) were conducted in batch reactors using pure cultures. The strains from contaminated sites were enriched and used as exogenous inoculums to remedy Cr (VI) contamination in the autoclaved soils. However, exogenous strains will inevitably lead to ecology risk in soil environmental due to the competition between exogenous strains and indigenous microorganisms. Since Cr (VI)-reducing microbial populations may widely distribute in soils, we can effectively utilize indigenous microorganisms to remedy contaminated soils. Furthermore, most

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of researches focus on the removal of water soluble Cr (VI). The information on the reduction of other Cr (VI) forms in soils is scanty.

In the present study, Cr (VI) biological reduction by indigenous microorganisms was investigated in contaminated soils by chromium-containing slag. In addition, the remediation of water soluble Cr (VI) and other Cr (VI) forms including exchangeable Cr (VI), carbonates-bonded Cr (VI), Fe and Mn oxides-bonded Cr (VI), organic matter-bonded Cr (VI) and residual Cr (VI) are also studied. The predominant strains that can reduce Cr (VI) were isolated and identified.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected from the top soil horizon (0-20 cm) of the sites under the chromium-containing slag heap at a steel-alloy factory in Hunan Province (27°75′N; 112°50′E) in central southern part of China. The samples were collected in spring, 2007. Ten kilograms of soil were taken, thoroughly mixed, air-dried and then passed through a 0.26 mm polyethylene sieve. The soil was classified as ferralic cambisol according to FAO/UNESCO soil classification system and the physical and chemical properties are presented in Table 1.

2.2. Nutrient medium

The nutrient medium for microorganism culture contained 10 g tryptone, 2 g NaCl, 10 g yeast extract, and 5 g sodium acetate in 1 L distilled water. The pH was adjusted to 9.8 by using either HCl or NaOH. All media were autoclaved at 121 °C for 30 min prior to use.

2.3. Cr (VI) remediation in contaminated soils

Ten grams of the contaminated soil was added into 10 mL of nutrient medium and incubated at 30 °C for 10 days. During the incubation, the moisture content of soil was adjusted every 2 days by adding deionized water equivalent to the loss of water. Soil samples were taken at 0, 1, 2, 3, 4, 5 and 10 days intervals, air-dried at room temperature, and then passed through a 0.26 mm polyethylene sieve. The sieved soil samples were used for determination of total Cr (VI), water soluble Cr (VI), exchangeable Cr (VI), carbonates-bonded Cr (VI), Fe and Mn oxides-bonded Cr (VI), organic matter-bonded Cr (VI) and residual Cr (VI). In parallel, control treatments without culture medium addition in the original soils and with culture medium addition in the autoclaved soils were also carried out to differentiate abiotic reduction of Cr (VI). In addition, in order to compare the effects of exogenous and indigenous microorganisms on Cr (VI) reduction, *Achromobacter* sp. CH-1, a Cr (VI)-reducing bacterium reported in our pervious research [17], was chosen to added into 10 g of autoclaved soils. The rest of the procedure was the same as described above for Cr (VI) remediation by indigenous microorganisms. All experiments were carried out from spring 2007 to spring 2008. Each treatment had three replicates.

2.4. Isolation of Cr (VI)-reducing bacterial strains

For isolation and enumeration of Cr (VI)-reducing bacterial strains, 10 g soil was added into 10 mL of nutrient medium and incubated at 30 °C for 4 days. Thereafter, the supernatant was decanted and diluted into a series of dilution sequences (1 mL supernatant was added into 9 mL double-distilled water). A 0.1 mL of 10^{-6}, 10^{-7} and 10^{-8} dilution sequence was spread on agar plates containing 250 mg L^{-1} Cr (VI). The bacteria were incubated at 30 °C for 5 days.

2.5. Cr (VI) reduction capacity by the isolates in pure cultures

A purified colony for each strain was inoculated into 100 mL of liquid nutrient medium and incubated on in a shaker with a speed of 150 rpm at 30 °C for 24 h. Thereafter, 10 mL of the grown bacterial cultures was transferred into 100 mL of fresh liquid nutrient medium containing 0, 25, 50, 100, 150, 200, 300, 400 and 500 mg L^{-1} Cr (VI), respectively, and incubated at 30 °C by shaking 72 h at a speed of 150 rpm. Aliquots of solution were withdrawn using a syringe at 0, 6, 12, 24, 36, 48, 60 and 72 h intervals and then divided into two portions. One portion was centrifuged and supernatants were analyzed for residual Cr (VI) concentration. The precipitates were collected, air-dried and then used to verify the oxidation state of chromium reduction product by X-ray photoelectron spectroscope (XPS). Another portion was used to evaluate cell growth by measuring optical density at 600 nm. A control treatment without bacterial strain inoculation was processed. All the experiments had three replicates.

2.6. Identification of the chromium-reducing strain

A single colony of chromium-reducing strain from a fresh plate was extracted using TIANamp Bacteria DNA Kit. Bacterial 16S rRNA gene primers 27R-5′CGGCTACCTTGTTACGACT3′ and 1502F-5′GAGTTTGATCCTGGCTCAG3′ were used for polymerase chain reaction (PCR) amplification of the 16Sr RNA gene. The PCR amplification was performed as following: each reaction was performed in a final volume of 50 μL, containing 25 μL 2 × mix, 1 μL each primer, 5 μL DNA sample, 18 μL deionised water. The reaction mixture was subjected to 30 cycles of amplification as follows: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. The amplified products were analyzed by electrophoresis on 1.2% agarose–TBE gels, stained with ethidium bromide (0.5 μg mL^{-1}) and visualized on a UV transilluminator. The PCR products were purified using the TIAquick Midi Purification Kit. The purified PCR products were sent to ShangHai Biological Company for sequencing. The sequences were initially analyzed at NCBI server (http://www.ncbi.nlm.nih.gov/) using BLAST tool and corresponding sequences were downloaded. The bacteria nucleotide sequences were aligned by using the CLUSTALX program. Phylogenetic tree was constructed by the neighbor-joining method using the MEGA program.

2.7. Analytical methods

The modified Tessier sequential extraction procedure was employed to determine the Cr (VI) fractions [16]. Briefly, water

Table 1
The physical and chemical properties of the tested soils.

Soil type	pH	OM (g kg^{-1})	CEC (cmol kg^{-1})	Particle size distribution (%)			Total Cr (VI) (mg kg^{-1})	Water soluble Cr (VI) (mg kg^{-1})
				①[a]	②	③		
Ferralic cambisol	9.8 ± 0.2	8.1 ± 0.6	13.2 ± 0.8	59.2 ± 9.1	21.6 ± 4.2	19.2 ± 3.6	462.8 ± 10.1	383.8 ± 9.8

OM, organic matter; CEC, cation exchange capacity.
[a] ①, >0.01 mm; ②, 0.01–0.001 mm; ③, <0.001 mm.

soluble Cr (VI) was extracted with distilled water at 1:8 ratio of soil to water for 30 min. Exchangeable Cr (VI) was extracted with 1 mol L^{-1} magnesium chloride for 1 h. Carbonates-bonded Cr (VI) was extracted with 1 mol L^{-1} sodium acetate solution (pH 5.0) for 6 h. Fe and Mn oxides-bonded Cr (VI) was extracted with 0.04 mol L^{-1} hydroxylamine hydrogen chloride in 25% (v/v) acetic acid for 6 h at 96 °C. Organic matter-bonded Cr (VI) was extracted with 0.02 mol L^{-1} nitric acid and 30% hydrogen peroxide at 85 °C for 3 h, extracted with an additional 30% H_2O_2 for 3 h at 85 °C (pH 2.0) and then extracted with 3.2 mol L^{-1} ammonium acetate in 20% (v/v) nitric acid for 30 min at room temperature. After each extraction, soil samples were centrifuged and Cr (VI) content in supernatants was determined using 1, 5-diphenylcarbohydrazide spectrophotometeric method (at 540 nm). The total Cr (VI) in soils was extracted by an alkaline digestion method (0.5 mol L^{-1} NaOH and 0.28 mol L^{-1} Na_2CO_3 at pH 11.5) and determined colorimetrically (λ_{540}) with a spectrophotometer. The difference between total Cr (VI) and water soluble Cr (VI), exchangeable Cr (VI), carbonates-bonded Cr (VI), Fe and Mn oxides-bonded Cr (VI) and organic matter-bonded Cr (VI) was regarded as residual Cr (VI).

2.8. Statistical analysis

An analysis of variance was carried out by the General Linear Model procedure of the SAS software package. Significant differences between means for different treatments were compared by means of the LSD test at $P < 0.05$.

3. Results and discussion

3.1. Cr (VI) remediation in the contaminated soils by indigenous microorganisms

In the contaminated soils under the chromium-containing slag heap in a steel-alloy factory, the average total Cr (VI) and water soluble Cr (VI) were 462.8 and 383.8 mg kg^{-1}, respectively. As shown in Fig. 1, total Cr (VI) concentration in original soils (non-autoclaved) without culture medium addition did not have significant change. Furthermore, nutrient medium addition in the autoclaved soil did not result in changes of total Cr (VI) concentration (data was not shown), indicating that abiotic matter revealed no ability of Cr (VI) remediation. However, when culture medium was added into the original soil (non-autoclaved), total Cr (VI) concentration declined from the initial value of 462.8 to 10 mg kg^{-1} at 10 days. It can be concluded that Cr (VI) remediation in the tested soils was contributed to Cr (VI) reduction by indigenous microorganisms. Similarly, when the original soils was inoculated with exogenous Cr (VI)-reducing bacterial strain, *Achromobacter* sp. Ch-1, the total Cr (VI) concentration in soils declined from an initial value of 462.8 to 7.6 mg kg^{-1} at 10 days (Fig. 1). There was no significant difference of total Cr (VI) concentration between *Achromobacter sp.* Ch-1 inoculation and indigenous microorganism. The result indicates that exogenous strain did not promote the reduction of Cr (VI) in the presence of indigenous microorganism. Hence, it is feasible to use indigenous microorganisms for Cr (VI) remediation in contaminated sites.

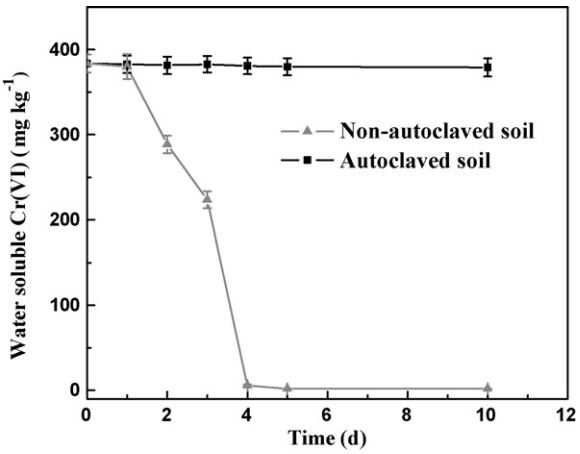


Fig. 2. Removal of water soluble Cr (VI) by indigenous microorganisms in the soils contaminated by chromium-containing slag.

The remediation of water soluble Cr (VI), exchangeable Cr (VI), carbonates-bonded Cr (VI), Fe and Mn oxides-bonded Cr (VI), organic matter-bonded Cr (VI) and residual Cr (VI) by indigenous microorganism in the contaminated soil were also investigated. The results presented in Figs. 2–4. As expected, in the autoclaved soils with culture medium addition, there was no significant decrease in concentrations of water soluble Cr (VI), exchangeable Cr (VI) and carbonate-bonded Cr (VI) during the 10 days incubation. However, once culture medium was added into the original soil (non-autoclaved soil), the concentrations of the above Cr (VI) forms decreased with prolonging incubation time. Water soluble Cr (VI) concentration declined from the initial concentration of 383.8 to 1.7 mg kg^{-1} after 10 days. Exchangeable Cr (VI) concentration decreased two folds within the first 2 days and it was removed by 92.6% at 10 days. Furthermore, carbonates-bonded Cr (VI) concentration decreased by 50% at 5 days and 6.8 mg kg^{-1} of carbonates-

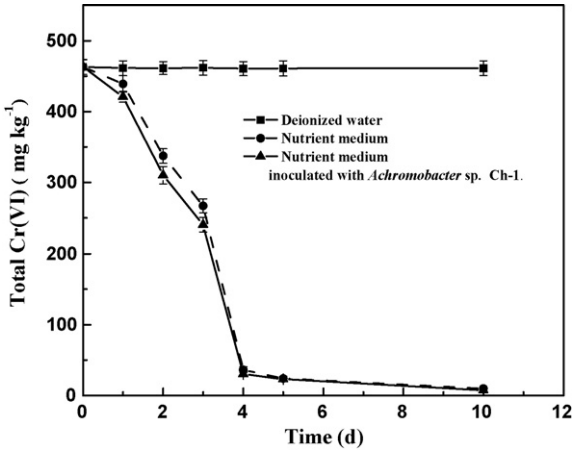


Fig. 1. Removal of total Cr (VI) in the soils contaminated by chromium-containing slag.

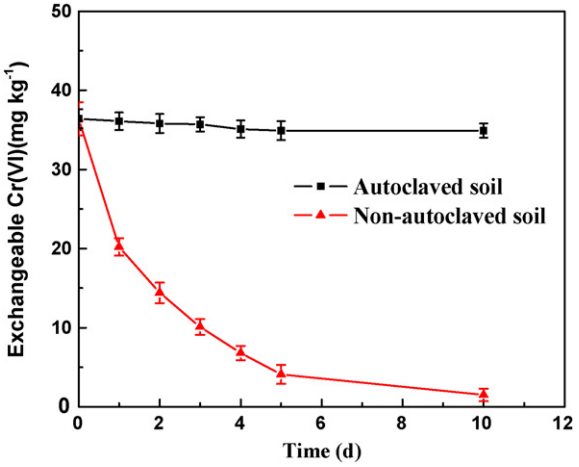


Fig. 3. Removal of exchangeable Cr (VI) by indigenous microorganisms in the soils contaminated by chromium-containing slag.

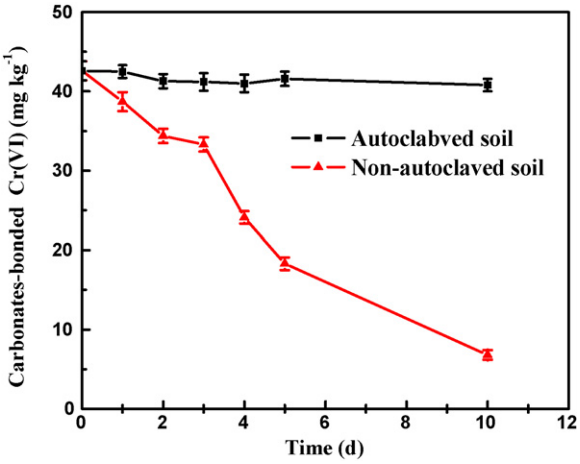


Fig. 4. Removal of carbonates-bonded Cr (VI) by indigenous microorganisms in the soils contaminated by chromium-containing slag.

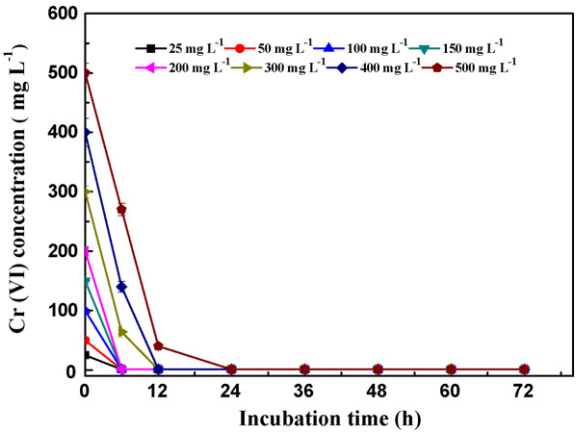


Fig. 5. Cr (VI) concentration at different initial concentrations by strain BB.

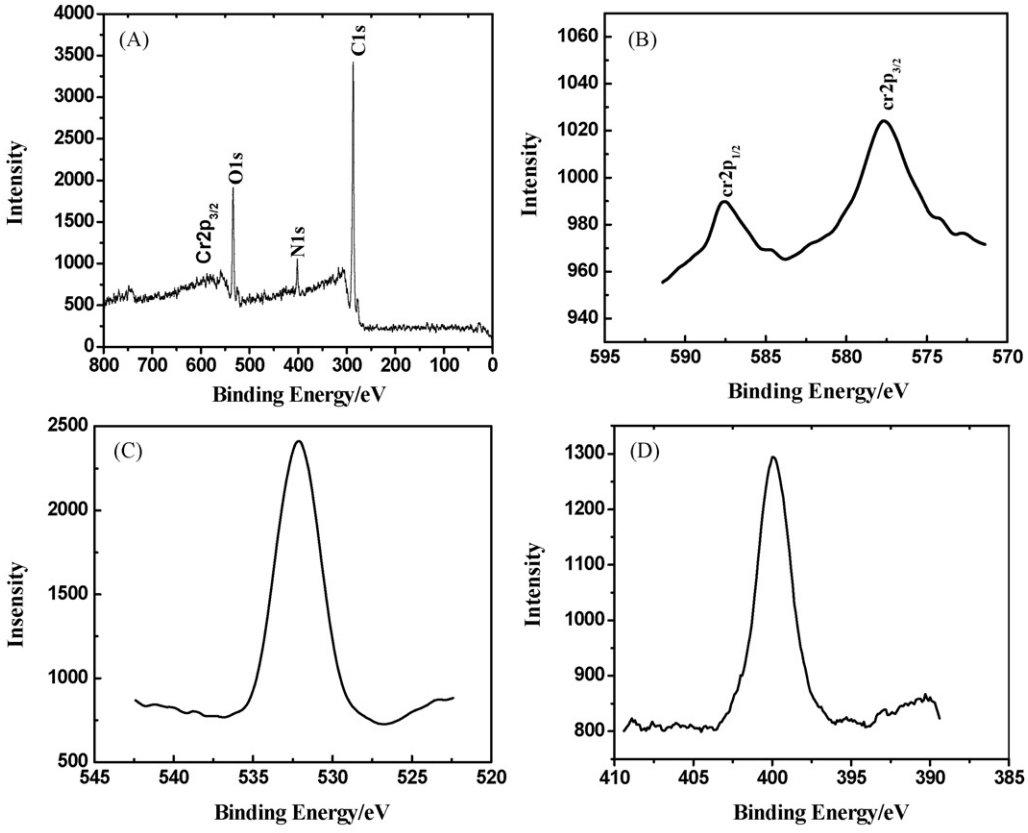
bonded Cr (VI) remained in the soils at 10 day. However, Fe and Mn oxides-bonded Cr (VI), organic matter-bonded Cr (VI) and residual Cr (VI) were not removed by indigenous microorganisms (data was not shown). The results revealed that indigenous microorganisms reduced not only water soluble Cr (VI), but also exchangeable Cr (VI) and carbonate-bonded Cr (VI) in contaminated soil.

3.2. Cr (VI)-reducing bacterial isolates

In order to obtain bacterial isolates, samples from a 4 days culture of contaminated soil were plated onto agar medium containing 250 mg L^{-1} Cr (VI). The agar plates were incubated at 30 °C for 5 days. Thereafter, bacterial colonies were separated and further purified. Four bacterial strains, representing the dominant Cr (VI)-resistant population, were isolated. These four strains showed obviously different color. The strains with blue, yellow, white and light grey color were named as BB, BY, BW and BG, respectively.

3.3. Cr (VI)-reducing capacity of bacterial isolates

The chromate-reducing abilities of bacterial isolates BB, BG, BY and BW were periodically monitored up to 72 hours at different initial concentrations of Cr (VI) ranging from 25 to 500 mg L^{-1} under aerobic condition. As shown in Fig. 5, strain BB reduced 200 mg L^{-1}



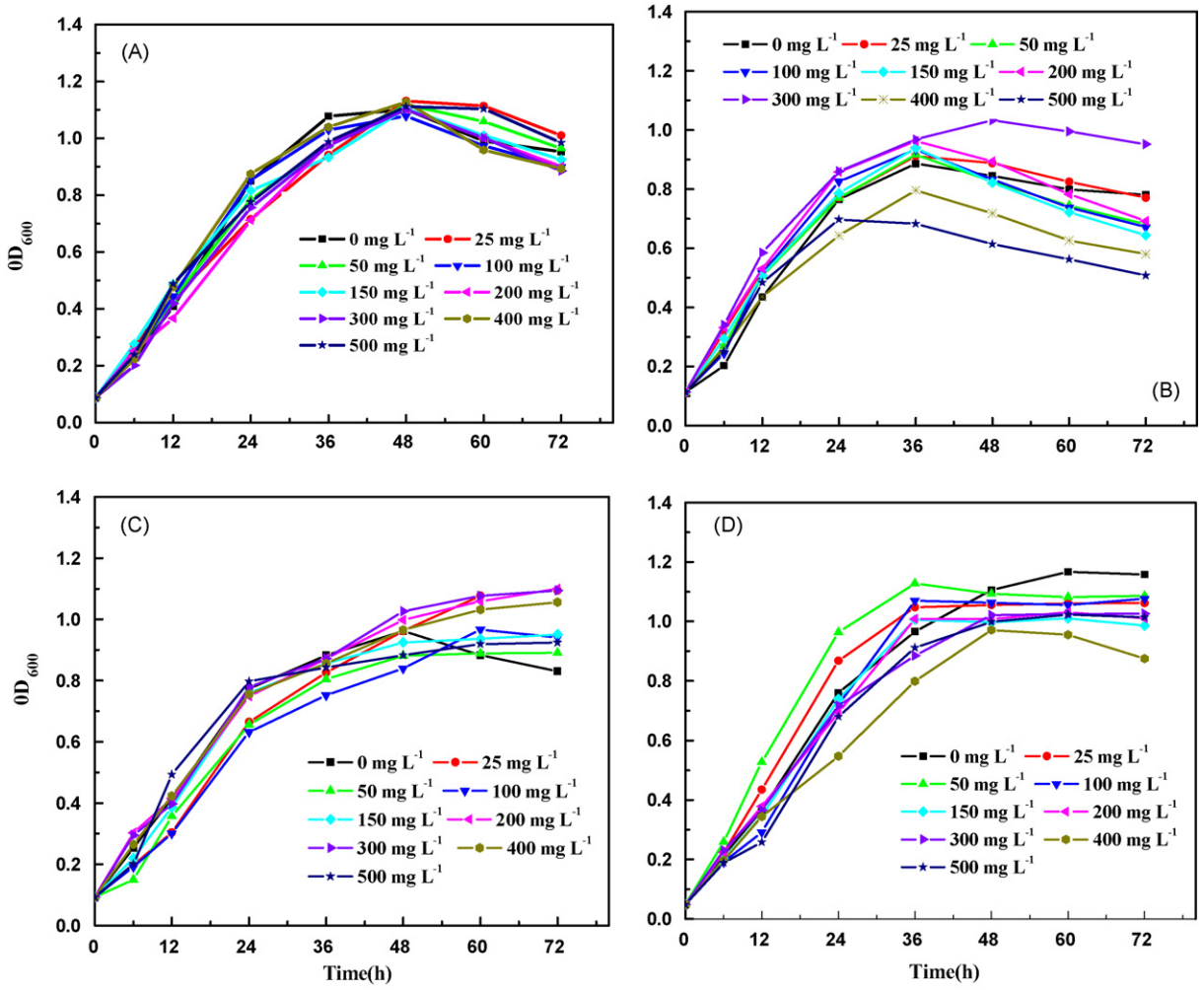
A: Full spectrum of elements; B: The Cr 2p spectra; C: The O is spectra; D: The N is spectra

Fig. 6. X-ray photoelectron spectra of Cr (VI) reduction product.

Cr (VI) within 6 h, and 300 and 400 mg L^{-1} Cr (VI) within 12 h. Even at high initial Cr (VI) concentration of 500 mg L^{-1}, Cr (VI) was almost completely reduced within 24 h. The results indicated that strain BB had a strong capacity for Cr (VI) reduction. However, the other three strains did not reduce hexavalent chromium at all the tested concentrations (data not shown). In this study, XPS was further employed to verify the oxidation state of Cr (VI) reduction product by the strain BB. As shown in Fig. 6(A), low resolution of XPS spectra of Cr (VI) reduction product indicated that Cr was the predominant element, while C, N and O were from cells components. Fig. 6(B) shows chromium spectrum in the product. The peaks of Cr 2p3/2 and Cr 2p1/2 in the spectrum were located at 577.1 ± 0.1 and 586.9 ± 0.1 eV respectively. The Cr 2p3/2 binding energies of Cr (III) hydroxides have been reported to be in the range of 576.5–576.9 eV and spin–orbit splitting for Cr (III) between the Cr 2p3/2 and Cr 2p1/2 peaks is around 9.9 eV, while the 2p3/2 peak for Cr (VI) has been shown between 579.0 and 579.8 eV and spin–orbit splitting for Cr (VI) is in the range of 8.7–9.4 eV [18]. The Cr 2p3/2 binding energies of $Cr(OH)_3{\cdot}4H_2O$ was located at 577.0 and 577.1 eV respectively in the reports of Asami and Hashimoto [19]. In Fig. 6(B), the peaks and their spin-orbit splittings demonstrated that hexavalent chromium was converted into trivalent as the form of Cr (III) hydroxides in the present study. Fig. 6(C) shows O 1 s spectra, from which the peak of O1S was located at 531.4 ± 0.1 eV. This binding energies represented metal–hydroxide (M–OH) bonds [20]. Therefore, the data from XPS patterns indicated that chromium species of the chromate reduction product was in the form of Cr (III) hydroxides. Furthermore, Cr (VI) peaks were not observed in the product indicating that Cr (VI) could be reductively transformed to Cr (III) and form Cr (III) hydroxides in alkaline condition by the strain BB.

In the previous literature, *Bacillus* sp., isolated from chromate contaminated soil, reduced Cr (VI) from 80 to 40 mg L^{-1} after 42 h in a nutrient medium, but Cr (VI) reduction ceased at higher contact times [21]. *Arthrobacter* sp., isolated from a long-term contaminated soil by tannery waste could reduced nearly 30 mg L^{-1} Cr (VI) during 46 h incubation and did not show any Cr (VI) reduction at 100 mg L^{-1} Cr (VI) during this incubation period [22]. *Burkholderia cepacia* MCMB-821 could reduced 98% of 75 mg L^{-1} Cr (VI) within 36 h at pH 9.0 [23]. *Providencia* sp. UTDM314 isolated from the contaminated sites of chemical industries could reduce 99.31% of 400 mg L^{-1} Cr (VI) within 100 h [24]. Similarly, *Ochrobactrum intermedium* SDCr-5 reduced 200 and 721 mg L^{-1} Cr (VI) within 72 and 96 h respectively [25]. *Microbacterium* sp. completely reduced 20 mg L^{-1} Cr (VI) within 72 h [26]. The *pseudomonad* strain CRB5 completely reduced 20 mg L^{-1} Cr (VI) within 120 h [27]. *B. sphaericus* AND303 did not completely removed 20 mg L^{-1} Cr (VI) even after 96 h [28]. Hence, strain BB exhibited higher ability for Cr (VI) reduction than the strains previously reported.

The effects of Cr (VI) on the growth of strain BB, BG, BY and BW in nutrient medium were also investigated by determining the



A: Strain BB; B: Strain BG; C: Strain BY; D: Strain BW

Fig. 7. Effect of different concentrations of Cr (VI) on the growth of four strains isolated from the contaminated soil.

OD_{600}. The growth responses of the four strains towards different Cr (VI) concentrations in nutrient medium are shown in Fig. 7. The results showed that the strains except for BG grew very well even at the concentration of 500 mg L^{-1} Cr (VI). There was no remarkable difference in curve patterns for each strain among the tested concentrations, indicating that the strains BB, BY and BW were tolerant to Cr (VI) concentrations as high as 500 mg L^{-1}. Nevertheless, the growth of strain BG was slightly inhibited after 24 h in the media containing 500 mg L^{-1} Cr (VI), elucidating that the threshold inhibitory concentration of Cr (VI) for this strain may be 500 mg L^{-1}. Anyhow, four strains revealed high Cr (VI) resistance as compared with strains reported by other researchers. The growth of *Bacillus subtilis* was significantly affected at 52 mg L^{-1} Cr (VI) and failed to grow at 104 mg L^{-1} Cr (VI) [29]. *Arthrobacter* sp. isolated from a long-term tannery waste contaminated soil could be tolerant to 100 mg L^{-1} Cr (VI) on a minimal salts agar medium [22]. *Pseudomonas* strains isolated from foundry soil were tolerant to 194 mg L^{-1} Cr (VI) in acetate minimal plates [30]. In the present study, complete Cr (VI) reduction at 200 mg L^{-1}, 300 and 400 mg L^{-1}, 500 mg L^{-1} by strain BB was achieved at 6, 12, 24 h respectively, while the maximal OD_{600} value was obtained at nearly 48 h. The result indicates that the strain growth can maintain a considerable period even after Cr (VI) reduction. Moreover Cr (VI) reduction ability of bacteria was growth independent (Figs. 5 and 7). The similar phenomenon was also found in the other three strains. Strain BY and BW did not show any Cr (VI) reduction even at low Cr (VI) concentration of 25 mg L^{-1}, but the growths of these bacteria were tenfold during this incubation period. These results further implied that Cr (VI) reduction ability of microbial consortium was not related to Cr (VI) resistance ability. Our results are in agreement with the work reported by Megharaj [22] who narrated that there was no relation between the growth of *Arthrobacter* sp. and its reduction of Cr (VI). Similarly, Klonowska et al. [31] found that the final biomass level of *D. vulgaris* ATCC 29579 was not affected at any Cr (VI) levels. Contrary result was also reported by Li et al. [32] who concluded that the rapid bacterial growth phase only occurred after complete Cr (VI) reduction.

3.4. Identification of isolates

The bacterial strain BB with the strong ability to reduce Cr (VI) was selected for identification using the 16S rRNA gene sequence. The sequence size was 1410 bp. This strain showed 99% similarity with *Pannonibacte phragmitetus*. The phylogenetic tree was shown in Fig. 8. On the basis of 16S rRNA gene sequence analysis, the isolate strain BB was identified as *Pannonibacter phragmitetus* sp. Several bacteria with a chromate-reducing ability were isolated from different contaminated sites in the previous literature, such as *Bacillus subtilis* sp., *Pseudomonas* sp., *Ochrobactrum intermedium* sp., *Micobacterium* sp., *Achromobacter* sp. [17,26,29,33,34]. However, so far, there was no report on the chromate reduction by *Pannonibacter phragmitetus*. This will provide a new microbial resource for microbial remediation of Cr (VI).

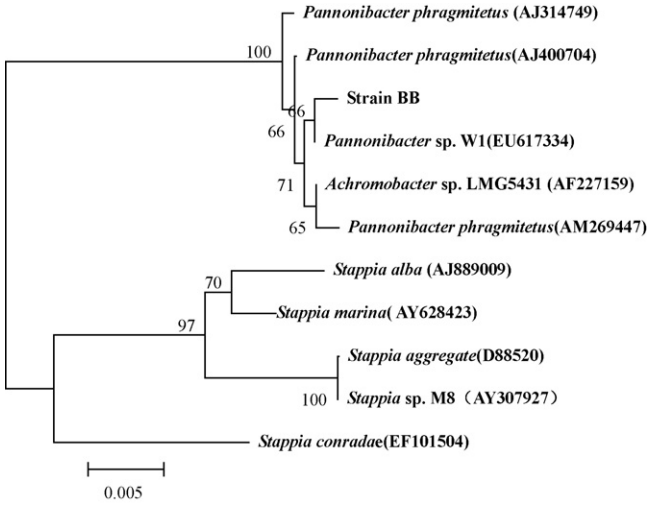


Fig. 8. Phylogenetic tree derived from 16S rRNA sequence data of strain BB and other related species. (The bar represents distance values. The values at nod represent percentage of 1000 bootstrap replicates. Numbers in bracket represent GenBank accession numbers.)

4. Conclusions

In the present study, indigenous bacteria consortium proved to be able to efficiently remediate Cr (VI) in the soils contaminated by chromium-containing slag. These indigenous bacteria not only effectively removed water soluble Cr (VI), but also removed exchangeable Cr (VI) and carbonates-bonded Cr (VI) within 10 days. Four indigenous bacteria strains were isolated from the Cr-contaminated soil. Only one strain had a high ability to reduce Cr (VI) and this isolate was identified as *Pannonibacter phragmitetus* sp. By 16S rRNA sequence. Therefore, *Pannonibacter phragmitetus* sp. could be responsible for the remediation of Cr (VI) in the contaminated soil. The results suggest that the use of indigenous bacterial populations provides a efficient and friendly environmental technique for biological remediation of Cr (VI) in the soils contaminated by chromium-containing slag.

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