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Original Research

Isolation and Characterization of Heavy Metal-Resistant Bacterias Capable of Removing Cr(VI)

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Abstract

A total of 53 strains of chromium-resistant bacteria were isolated from Meiliang Bay of Taihu Lake, China and were tested for Cr(VI) resistance. The strain THKCS311 with the maximum growth value under Cr(VI) stress was regarded as the optimal strain for further study. The partial sequences were amplified from the strain and the BLAST query revealed that it was closely related to *Bacillus* sp., and it had 98% homologous to *Bacillus mycoides* strain 273 and *Bacillus anthracis* strain ATCC 14578. Batch experiments were conducted to remove Cr(VI) using THKCS311, and the effects of the initial Cr(VI) concentration, pH, and temperature condition on Cr(VI) removal efficiency were investigated. The results showed that *Bacillus* sp. can mediate reduction of Cr(VI)-Cr(III), and the removal efficiency decreased with the increase of initial Cr(VI) concentration. The removal efficiency of Cr(VI) was highest at pH 6.5 and 35ºC, and removal efficiencies were 59.2% and 60.7%, respectively. SEM micrographs indicated that THKCS311 cells were irregular and cracked with the appearance of wrinkles on the surface after Cr(VI) stress.

Keywords: chromate reduction, bacteria, Cr(VI), THKCS311

Introduction

Low concentrations of certain transition metals such as cobalt, copper, nickel, and zinc are essential for many cellular processes of bacteria. However, higher concentrations of these metals often are cytotoxic. Other heavy metals, including lead, cadmium, mercury, silver, and chromium have no known beneficial effects to bacterial cells and are toxic even at low concentrations [1]. Chromium compounds are used extensively in numerous industrial processes such as leather-tanning, metal plating, and finishing, wood treatment, corrosion inhibition in power plants and nuclear facilities, and in the manufacturing of refractory materials, pigments, dyes, textiles, and mining equipment, among others [2].

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Chromium exists in oxidation states ranging from 0 to +6, among which Cr(III) and Cr(VI) are commonly observed in environmental samples [3]. In contrast to Cr(VI), Cr(III) is less mobile and forms water insoluble compounds in aqueous solution. Cr(III) is less toxic than Cr(VI), therefore conversion of Cr(VI) to Cr(III) is an effective way of combating Cr(VI) pollution. Cr(VI) is highly soluble and is easily taken up by cells [4]. Inside the cells it is reduced partially to highly unstable Cr(V) radical, which leads to the formation of reactive oxygen species and the oxidative stress thus generated is the cause of carcinogenicity [5].

Conventional technologies for Cr(VI) removal include chemical reduction, precipitation, ion exchange, membrane separation, and adsorption [6]. Increasing attention has been paid recently to the use of eco-friendly and low-cost biomaterials for Cr removal from wastewater, such as

bacteria, fungi, algae, and industrial and agricultural wastes [7-10]. A number of bacterial species have been isolated and shown to be capable of Cr(VI) reduction. These microorganisms have developed the capabilities to protect themselves from heavy-metal toxicity by various mechanisms such as adsorption, uptake, methylation, oxidation, and reduction. Many microorganisms have been reported to reduce the highly soluble and toxic Cr(VI) to the less soluble and less toxic Cr(III), e.g., *Acinetobacter* and *Ochrobactrum* [11], *Arthrobacter* [12], *Ochrobactrum* sp. [13], *Bacillus* sp. [14], *Cellulomonas* sp. [15], *Bacillus* sp. [16], *Sporosarcina* sp. [17], and *Bacillus* sp. [18].

The present study deals with the isolation of chromium-resistant bacteria from a contaminated environment, their molecular characterization, the ability of the bacteria to reduce hexavalent chromium, and the optimization of initial chromium concentrations, temperature, and pH for Cr(VI) removal efficiency. In addition, SEM experiments were carried out to obtain cell surface changes after Cr(VI) stress.

Materials and Methods

Sediment Samples

Sediment samples were collected from Meiliang Bay of Taihu Lake, China, in July 2013. The area was contaminated with chromium from nearby industrial activities. Total Cr concentration in the sediments was 100 mg/kg.

Isolation and Selection of Optimal Cr(VI) Resistant Strain

Heterotrophic bacteria were isolated by using R_2A agar medium. Briefly, 1 L of double-distilled water containing 0.5 g yeast extract, 0.5 g protease peptone, 0.5 g casamino acids, 0.5 g dextrose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g KH_2PO_4, 0.05g $MgSO_4$ and 15 g agar (pH 7.2 0.2). The solution was suspended and kept in autoclave at 121ºC for 20 minutes, and the mixture was poured onto the plate after cooling to 55ºC.

To isolate Cr(VI)-resistant strains, the samples were screened on R_2A plates and supplemented with Cr(VI) at 100 mg/L. We added 100 µL sediment slurry to the R_2A plates, coating uniformity, and then incubated the cultures at 35ºC for 1-3 d.

In order to select the best heavy metal-resistant strains, we numbered the strains 1-53 and then inoculated them into 5 ml TY liquid medium (5 g protease peptone; 3 g yeast extract; 5 g NaCl; and 1g glucose per liter), supplemented with 100 mg/L Cr(VI). The cultures were incubated at 35ºC and agitated at 150 rpm for 60 h. The growth values of the strains were determined by absorbance at 600 nm (OD600). All tests were done in triplicate.

Identification of the Selected Strain

The selected strain was identified using molecular methods, and the 16SrRNA gene was amplified by PCR using two general bacterial 16SrRNA primers: 1492R and 27F (27F 5' AGA GTT TGA TCM TGG CTC AG 3'; 1492R 5' TAC GGY TAC CTT GTT ACG ACT T 3'). The PCR conditions used were: an initial denaturation step at 94ºC for 3 min, followed by 30 cycles at 94ºC for 1 min, 56ºC for 1 min, 72ºC for 2 min, and a final extension step at 72ºC for 10 min [19]. PCR products were detected by electrophoresis on 1% TAE agarose gel, stained with ethidium bromide, and visualized using a BioRad UV transillumination (UVP EC3, USA). Sequencing was carried out by Shanghai Shenggong Company, China. The 16SrRNA gene sequences were compared with known sequences in the GenBank database to identify the most similar sequence alignment. The nucleotide sequences of 16SrRNA gene were aligned and a phylogenetic tree was constructed with Mega 5.1 software using the neighbor-joining method.

Effects of Growth Conditions on Cr(VI) Reduction

In experiments to evaluate the effects of the initial Cr(VI) concentration, initial concentrations of 12.5-100 mg/L were used. In experiments to determine the effects of pH, individual culture media under aerobic conditions were adjusted to pH 4.5, 5.5, 6.5, 7.5, and 8.5 by the addition of 0.1M HCl and 0.1M NaOH. In experiments to compare the effects of different growth temperatures, the following temperatures were tested as a growth condition: 25ºC, 35ºC, and 45ºC. In order to obtains the Cr(VI) and Cr(III) concentration variation in the treatment solution, experiments were performed under 100 mg/L Cr(VI), pH 6.5 and 35ºC.

SEM Observations

SEM analysis was performed on an environmental SEM (HITACHI S-4800), the analytical conditions varied as follows: backscattered electrons mode (BSE), magnification of 10,000 times, electron beam voltage of 3.0 kV, work distance of 8.5 mm, and temperature of 20ºC. The bacteria samples were examined after cell fixation and vacuum freeze drying.

Chromium Analysis

The concentrations of total Cr in the liquid samples were determined by ICP-MS using Agilent 7700, USA). The concentrations of Cr(VI) were analyzed by measuring the absorbance of the purple complex of Cr(VI) with 1,5-diphenylcarbohydrazide at 540nm by a UV spectrophotometer (UV3600, Shimadzu, Japan). The concentrations of Cr(III) were then obtained from the difference between total Cr and Cr(VI) concentrations.

Results and Discussion

Selecting the Optimal Cr(VI)-Resistant Strain

A total of 53 Cr(VI)-tolerant bacterial strains (THKCS 1-53) were isolated from Meiliang Bay using R_2A agar

medium supplemented with 100 mg/L Cr(VI). The growth values of Cr(VI)-resistant strains determined by absorbance at 600 nm are shown in Fig. 1, No. 31 strain, named THKCS311 with the maximum growth value (1.467) was regarded as the optimal strain for further study.

16SrRNA Phylogenic Analysis

Sequence analysis of the 16SrRNA gene is a fast and accurate method to identify the phylogenic position of bacteria. The partial 16SrRNA sequences of THKCS311 were uploaded to the National Center for Biotechnology Information (NCBI) website to search for similarity to known DNA sequences and to confirm the species of this local isolate. The BLAST query revealed that it was closely related to *Bacillus* sp., and it had 98% homologous to *Bacillus mycoides* strain 273 and *Bacillus anthracis* strain ATCC 14578. A phylogenetic (neighbor-joining) tree was constructed and is shown in Fig. 2.

Effect of Initial Cr(VI) Concentrations on Cr(VI) Reduction

The effect of initial chromium concentration on Cr(VI) reduction by THKCS311 was studied over a range of Cr(VI) concentrations from 12.5 to 100 mg/L. The removal efficiency of Cr(VI) by resistant strain THKCS311 in solution was calculated from the differences between the removal efficiencies of Cr(VI) of inoculated medium and non-inoculated medium. This Cr(VI) reduction in the enrichment culture medium indicates a microbiological process. As illustrated in Fig. 3, when the initial concentration of Cr(VI) was 12.5 mg/L, almost complete reduction of Cr(VI) was achieved after 48h. In the case of a 25 mg/L concentration of Cr(VI), 82.19% of the initial Cr(VI) was removed in 48h. When the initial concentration of Cr(VI) was 50 mg/L and 100 mg/L, 66.2% and 57.9% of the dissolved Cr(VI) was reduced in the same period. The removal efficiency of Cr(VI) by THKCS311 decreased with an increase of initial Cr(VI) concentration in the enrichment culture medium.

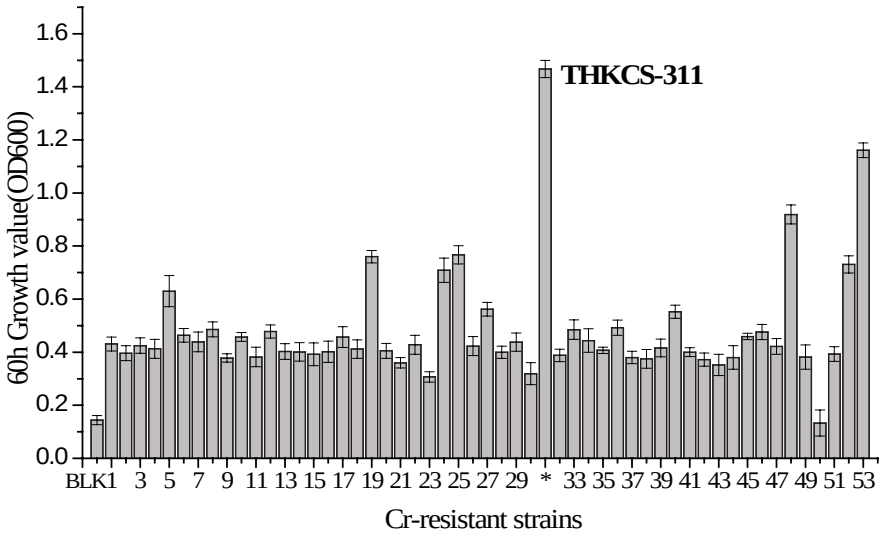

Fig. 1. Growth value of Cr-resistant strains in the presence of 100 mg/L Cr(VI).

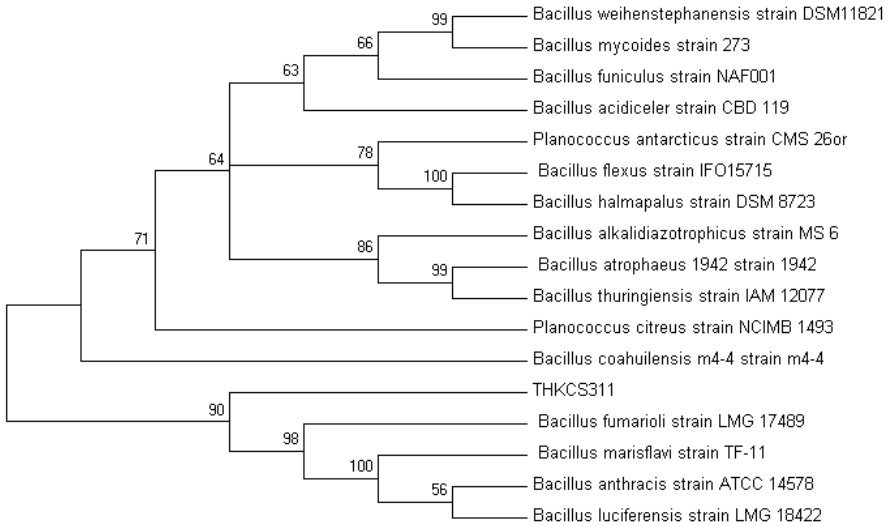

Fig. 2. Phylogenetic relationships by a neighboring analysis of the 16SrRNA sequences showing the position of the strain THKCS311.

Effects of the pH on Cr(VI) Reduction

pH is one of the most important factors influencing chemical speciation, solubility, and bioavailability of Cr in the field [20]. The variation in pH of the medium causes changes in the ionic form of the active site of the chromium reduction enzyme and affects its activity. pH range 6-8.5 was found to be optimum for Cr(VI) reduction by most of the bacterial strains [21].

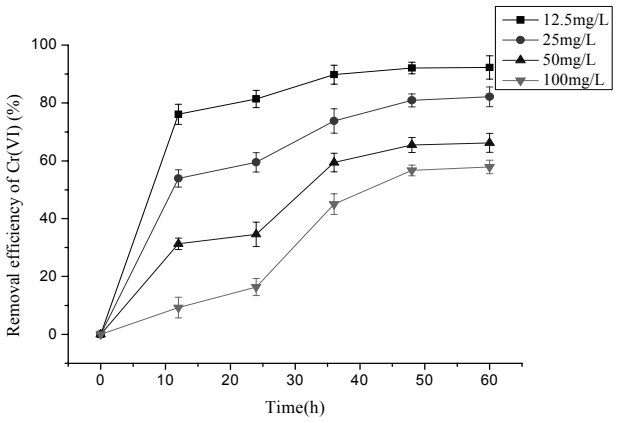

Fig. 3. Removal efficiency of Cr(VI) by THKCS311.

The effect of pH on Cr(VI) reduction in the enrichment culture medium was shown in Fig. 4, with initial Cr(VI) concentration in the enrichment culture medium set at 100 mg/L. At pH 6.5 the removal efficiency of Cr(VI) was greatest, 59.2% of the initial Cr(VI) was reduced within 48h. At pH 8.5 the removal efficiency of Cr(VI) was the least, and 33.7% of the initial Cr(VI) was reduced within 48 h. In the range of pH 4.5, pH 5.5, and pH 7.5, 39.3%, 48.7%, and 45.7% of the initial Cr(VI) was reduced in the same period.

Effects of Temperature on Cr(VI) Reduction

Effects of temperature on Cr(VI) reduction in the enrichment culture medium are shown in Fig. 5, with initial Cr(VI) concentration in the enrichment culture medium set at 100 mg/L. When the culture temperature was 25ºC, 45.8% of the dissolved Cr(VI) was reduced in 48h. In the

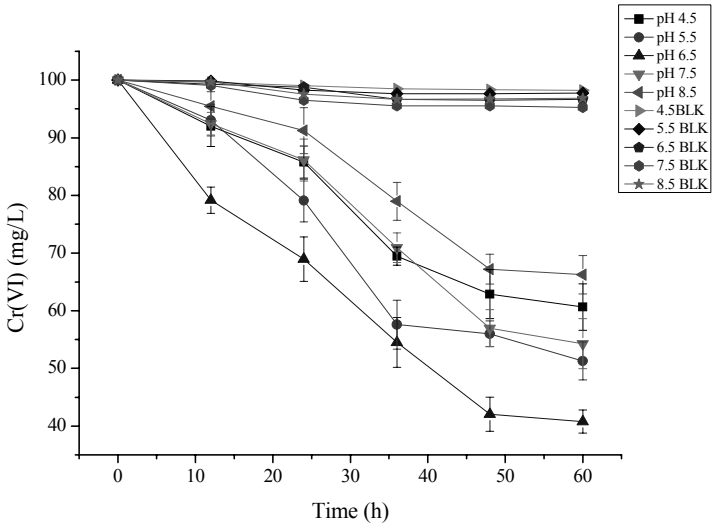

Fig. 4. Effects of pH on Cr(VI) reduction.

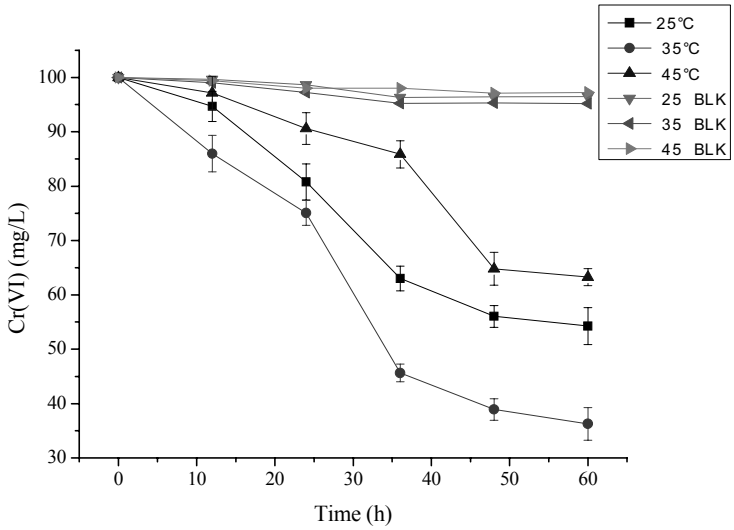

Fig. 5. Effects of temperature on Cr(VI) reduction.

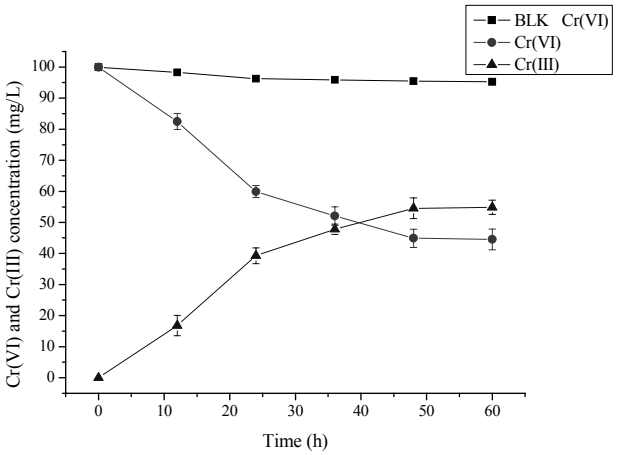

Fig. 6. Cr(VI)and Cr(III) concentration variations in the treatment solution.

case of a culture temperature at 35°C, 63.7% of the initial Cr(VI) was removed in 48h. When the culture temperature was 45ºC, 36.7% of the dissolved Cr(VI) was reduced in the same period.

The temperature of 35ºC was the optimum temperature for Cr(VI) reduction by THKCS311. Extreme temperatures severely reduced bacterial growth and chromate reduction due to loss of viability or metabolic activity of cells on prolonged incubation. At temperatures higher than optimum, loss of chromium reduction function, alteration of membrane structure, or inactivation of protein synthesizing mechanism due to alteration of ribosome conformation takes place. At low temperatures, the fluidity of the membrane decreases sufficiently, which prevents the functioning of the transport systems so that the substrates cannot enter into the cell rapidly, to support even low rate of growth [22].

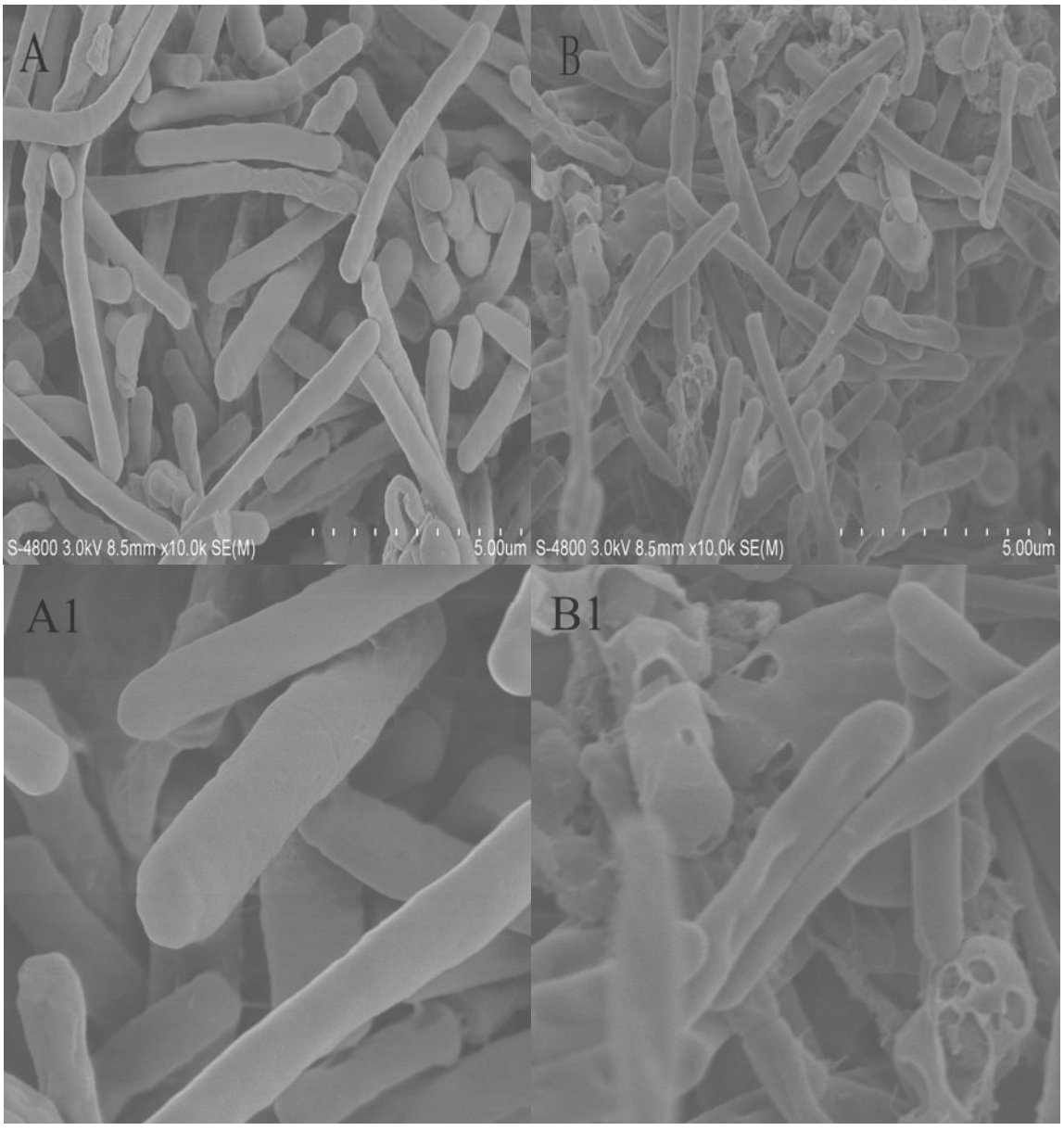

Fig. 7. Scanning electron micrographs of THKCS311 cells grown on TY medium (A, A1) without Cr(VI) (control) and (B, B1) with 100 mg/L Cr(VI).

Cr(VI) and Cr(III) Concentration Variations in the Treatment Solution

From Fig. 6 we notice that the Cr(VI) concentration decreased rapidly and Cr(III), which initially did not exist, and its concentration increased proportionally to the Cr(VI) depletion. These results indicated that Cr(VI) was reduced to Cr(III) when contacting with the bacteria and some of the converted Cr(III) released into the aqueous phase. The possible mechanism could be the surface enzymatic reaction of Cr(VI) by the bacteria to Cr(III). The reduced chromium was mostly coordinated with the functional groups on the bacterial surface or partially released into the supernatant [23].

The removal procedure was supposed to involve sorption and reduction, which may go through certain stages:
(1) Sorption of Cr(VI) onto the bacteria surface
(2) Reduction of Cr(VI) to Cr(III) by surface functional groups
(3) Release of the converted Cr(III) from, or sorption to various functional groups of the bacteria, depending on environmental factors [24].

SEM Observations of THKCS311

SEM micrographs obtained from THKCS311 grown without Cr(VI) (control) and exposed to 100 mg/L Cr(VI) are presented in Fig. 7. The SEM micrographs showed that THKCS311 grown without Cr(VI) appeared as coccobacilli (Figs. 7. A, A1) with smooth surface. The average diameter of the cells was approximately 0.5 μm. However, after 24 hours of growth on TY liquid medium containing 100 mg/L of Cr(VI), the morphology of THKCS311 cells (Figs. 7. B, B1) were irregular and cracked with the appearance of wrinkles on the surface.

Conclusions

In this study the Cr(VI)-resistant strains isolated from Cr-contaminated sediment were investigated for their effects on Cr(VI) reduction with respect to the initial Cr(VI) concentration, temperature, and pH condition. The results of the present study indicated that Cr(VI)-resistant strain THKCS311 was closely related to *Bacillus* sp., and it could successfully remove reduction of Cr(VI) to Cr(III), the removal efficiency can reach above 60 percent in 48h. Low Cr(VI) concentration, suitable pH, and temperature condition could enhance the removal rate. Through SEM micrographs we can know that THKCS311 cells were irregular and cracked with the appearance of wrinkles on the surface after Cr(VI) stress.

The properties of the Cr-resistant bacteria, which can reduce Cr(VI), make them potentially useful for the bioremediation of Cr-contaminated sediments, but the complete mechanism of Cr(VI) reduction still needs further research.

Acknowledgements

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