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Uranium (VI) bioremediation by Acinetobacter sp. USC B2 isolated from uranium tailings area

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Abstract. Bioremediation of uranium (VI)-contaminated aquifers was investigated by using bacteria isolated from a uranium tailing in South China. The bacteria were identified as Acinetobacter on the basis of physiological, biochemical tests and 16S rDNA sequence homology analysis. The protein concentration in the supernatant declined from 50.26 μg/mL to 40.01 μg/mL after uranium removal, and the activity of bacterial phosphatase increased from 36.04 U/L to 39.81 U/L. The FTIR, SEM-EDS and XRD demonstrated the involvement of functional groups in U (VI) binding, U accumulated on the surface of the bacteria and the precipitated is Uranyl Sulfite Hydrate. The results from this study demonstrate the potential of the U tolerant, bacterium Acinetobacter sp. USC B2 isolate might be considered as promising candidates in the design of uranium bioremediation strategies.

1. Introduction

With the development of nuclear science and technology, it has led to an increase in the incidence of uranium contamination of soil, sediment and groundwater with uranium (U) become a topic of serious environmental concern around the world [1,2]. Especially, improper disposal of waste and wastewater containing these elements from various activities causes accumulation of these compounds in surface and groundwater systems. Complete removal of U from groundwater and soil by physical or chemical methods is feasible on large spatial scales and concurrently generates secondary waste streams [3]. Alternatively, microbial bioremediation offers some expensive, in situ technique, especially in the remediation of U contaminated areas [4].

The bacteria are known to influence the mobility of U and other radionuclides by (1) reductive precipitation of U(VI) to U(IV) [5,6], (2) U(VI) biosorption and accumulation by cells [7,8], and (4) biomineralization of U(VI) with phosphates, carbonates [9,10]. Mainly found in the form of hexavalent ions in aqueous systems, it reacts with two “oxo” oxygen ligands, the interactions between protein and uranium can be described by the constant formation of a protein–uranium complex [11].

A determined understanding of mechanisms of microbial transformations of uranium under water environmental conditions will be valuable in developing appropriate remediation and waste management strategies. The aim of this study is to utilize situ cultures of bacteria from the local environment to bio precipitation U(VI) and the objective was to investigate the ability of the pure bacteria and identified as Acinetobacter sp. USC B2. Furthermore, the mechanism of bacteria and
U(VI) was described by means of determination of the changes of phosphatase activity in the bacteria and provide reference for the biological repair of uranium.

2. Materials and methods

2.1. Isolation of bacteria and physiological-biochemical characteristic
26 bacterial strains, previously isolated from a uranium tailing soil in southern China and maintained in our laboratory culture collection, were inoculated into 100 mL LB broth medium(sigma) and incubated at 30°C on an orbital shaker for 24 hours. Four milliliters of the LB medium culture was inoculated into 96 mL of new LB medium culture at pH 7, containing 10 mg/L U(VI). Physiological and biochemical tests were carried out on the resistant of bacteria to U(VI) toxicity, and the physiological and biochemical characteristics of the dominant bacteria were identified by oxygen, temperature, pH and carbon source. The growth morphology, size, color and growth status of bacteria on the surface and edge of microorganism in solid culture medium were observed. The bacteria were observed by gram staining method, and the basic morphological structure and size and arrangement of bacteria were observed by microscopy.

2.2. 16S rDNA sequence identification
For the isolate, nearly the complete small ribosomal subunit 16S rDNA gene (c.1500 bp) was amplified. The products were bi-directionally sequenced using the same primers used during the amplification step Qing ke biotechnology co., LTD. All sequences were compared with those available in the GenBank databases by BLAST. 16S rDNA gene sequence obtained in the study was deposited in the database under accession number MF083941.

2.3. Protein extract, concentration and enzyme activity
The protein extraction was carried out by the hyperbiosis method. Protein concentration was determined in a UV/Vis spectrophotometer (WPA, Light Wave II) at the wavelength of 595 nm using Coomassie brilliant blue G-250 dye as a complexing agent to facilitate protein detection. Phosphatase activity was measured with disodium phenyl phosphate assay. A fixed-volume sample was added to 4-Aminoantipyrine (0.1M, pH 10), disodium phenyl phosphate (0.02M) and Potassium ferricyanide. The absorbance at a wavelength of 510 nm was measured and compared to a set of similar-volume standards.

2.4. mechanism analysis
The samples were obtained by vacuum freeze-drying. FT-IR was performed to identify the functional groups on the cell surface. The spectra were recorded within the range of 4000–400 cm⁻¹ with samples prepared as KBr discs.

The surface morphology of the samples was analyzed by SEM (JEM-7600F, Joel, Tokyo, Japan) at 10 kV accelerating voltage. The samples for SEM observations were prepared by coating with a thin film of gold before measuring. In additional, elemental analysis of the collected samples was conducted by EDS equipped on the JEM-7600F.

XRD analysis was carried by Rigaku Miniflex 600 at a power of 35 kV and 35 mA. The diffraction spectra were obtained by scanning the sample in the range of 5°-80° in steps of 0.02°.

3. Results and discussion

3.1. Isolation of bacteria and physiological-biochemical characteristic
Almost all the culture media used results in bacterial growth. Five bacterial isolates among the 26 strains showed high tolerance of uranium in the LB-agar. A strain showed good removal of uranium: 9.26–9.53 mg L⁻¹ in initial concentration of 9.6 mg L⁻¹. The strain was subjected to physiological and biochemical parameters, it was identified as aerobic bacteria and the growth rate of bacteria increased
with the increase of temperature at 4–30 °C, reached the maximum at 30°C, decreased at 37°C, ceased to grow at 60°C; The bacteria can grow at different pH conditions, the best growth at pH = 7, through different carbon source culture found that bacteria can be in glucose and sodium lactate grow well.

The 16S rDNA gene sequences obtained were used to perform BLAST search against the GenBank and EzTaxon-e Type Strains databases to identify their closest matches. The isolate sample was assigned up to genus or species level, by their values of sequence similarity, higher than 99% (Table 1). Among the identified microbial strain was affiliated to Acinetobacter.

Table 1. The results of 16S rDNA sequence of dominant strains

| Description of similar strains                  | Ident | Max score | Accession |
|------------------------------------------------|-------|-----------|-----------|
| Acinetobacter Johnsonian strain_261ZY15         | 99%   | 1448      | KF831405  |
| Acinetobacter Johnsonian strain_YH16108         | 99%   | 1423      | KY767497  |
| Acinetobacter_sp._enrichment_culture_clone_WT3  | 99%   | 1433      | GQ369439  |
| Acinetobacter_johnsonii_strain_SP17             | 99%   | 1440      | JN409466  |
| Acinetobacter_johnsonii_strain_LCG40           | 99%   | 1409      | KY643718  |
| Acinetobacter_sp._JB-5                         | 99%   | 1426      | KP265957  |

3.2. Protein extract, concentration and enzyme activity

In order to obtain reliable evidences for bioremediation, the concentration of protein was performed for the bacterial strain isolated. It was found that the total protein concentration was changed from 50.26 μg/mL to 40.01 μg/mL under uranium-treated and uranium content changed from 9.6mg/L to 3.76mg/L, the removal rate was 62.45%. It can be seen that the total protein of bacteria reacts with uranium in solution, formed precipitation, reduced the soluble uranium content while decreased the soluble protein content.

Presence of enzymatic activities was assayed for the microbial. In the case of phosphatase activity, relevant in uranium biomineralization processes, the strains displayed positive results: the activity of Acinetobacter was increased from 36.04 U/L to 39.81 U/L after uranium removal. This may be the participation of phosphatase in the reaction of uranium with bacteria, and phosphatase reacts with uranium to promote the secretion of phosphatase and improve the activity of phosphatase.

3.3. Analysis

3.3.1. FTIR spectroscopy. The paragraph text follows on from the subsubsection heading but should not be in italic. When receiving the paper, we assume that the corresponding authors grant us the copyright to use the paper for the book or journal in question. Should authors use tables or figures from other Publications, they must ask the corresponding publishers to grant them the right to publish this material in their paper.

FTIR spectroscopies for control (metal-free) and for uranium a loaded biomass was recorded to elucidate the chemical groups involved in metal U binding on to the bacterial biomass (Fig.1). In the control spectrum, a peak in the 3306 cm⁻¹ region is due to the stretching of the N-H bond of amino groups. This N-H stretching peak lies in the spectrum region occupied by a broad and strong band (3200–3600 cm⁻¹), which is due to the presence of O–H of the hydroxyl groups [12]. The absorption peak at 1650cm⁻¹ is the C=O bond of the amide I band on the protein, the absorption band at the 1533cm⁻¹ is the amide II band, which is caused by the N-H bond bending vibration and the C-N bond stretching vibration of the secondary amide, and uranium After the effect, there were no significant changes in the two peaks of the characteristic bands of the protein, but the peak intensity was weakened, indicating that the characteristic bands of the protein played a role in uranium. The absorption peak at 1388cm⁻¹ shifted to 1405 cm⁻¹ after uranium was due to COO-symmetrical stretching vibration, indicating that carboxyl participates in the reaction of uranium and participates in the reaction of uranium. Absorption peak at 1069 cm⁻¹ May nucleic acid and ATP dihydrogen phosphate ester groups of symmetric and anti-symmetric stretching vibration peak, in addition to the
uranium after narrow peak peak shape into a 1004 cm$^{-1}$, the change of the position and intensity could be due to P-O involved in uranium deposits [13]. FTIR spectroscopic analysis illustrated the amide, hydroxyl, carboxyl, and phosphate groups were main functional groups in uranium interaction.

![FTIR spectra](image)

**Fig. 1.** Fourier transformed IR (FTIR) spectra of *Acinetobacter* sp. USC B2 biomass: before (a) and after uranium (b) accumulation

![SEM-EDS](image)

**Fig. 2.** Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) spectrum of *Acinetobacter* sp. USC B2: before (a) and after uranium (b) accumulation.

3.3.2. **SEM-EDS.** To establish the distribution and localization of uranium binding site on the cells, the cells exposed to U(VI) were viewed using scanning electron microscopy (SEM), to observe the changes of the bacteria (Fig.2). it can be seen that the volume of *Acinetobacter* bacteria is significantly larger, the surface is no longer flat, and there are obvious clusters of aggregates on the surface of bacteria. The results of EDS showed that the U peaks appeared on the aggregates of aggregates and bacteria, and the P peaks also increased. During the process of the yeast and uranium reported in the literature, nanosized UP crystallized mineralized bodies were generated on the surface of bacteria [14].
The mechanism of adsorption of uranium with Deinococcus radiodurans is significantly different [15]. This study shows that the mechanism of action of Acinetobacter and uranium is similar to yeast.

3.3.3. XRD analysis. Uranium loaded biomass showed distinct peaks in XRD (Fig.3) also indicating crystallinity within the sample. The XRD pattern of this sample showed distinct peaks at 20, 17.79 and 26.25 corresponding to D-spacing values of 4.91 Å, and 3.99 Å, respectively. A comparison of these d-spacing values with data files of known compounds (JCPDS), showed the satisfactory correlation with the lines of U phosphate compounds saleeite \([UO_2]_2(PO_4)_2\cdot4H_2O\) and uranyl phosphate \([UO_2(PO_3)_2]\). The phosphatase of bacteria is proved to be involved in uranium precipitation, and uranyl precipitation is formed.

![XRD spectrum](image)

**Fig. 3.** X-Ray Diffraction (XRD) spectrum of *Acinetobacter* sp. USC B2: before (a) and after uranium (b) accumulation.

4. Conclusion
In conclusion, our study about identification and characterization of natural U-tolerant bacteria may be considered as baseline information for future studies focused on utilization of autochthonous microbes in bioremediation strategies. Our results demonstrated the bacterium Acinetobacter sp. USC B2 isolated from uranium tailing exhibited inherent properties of uranium bio precipitation. The content of bacterial protein was reduced after uranium added and the activity of phosphatase increased. Analysis of the precipitate of bacteria by FTIR indicated the involvement of functional groups like the amide, carboxyl, hydroxyl, and phosphate in facilitating precipitation of uranium. The SEM-EDS demonstrated that the surface of the bacteria and the content of the element were changed, it’s indicated that the bacteria and uranium have been exchanged to promote the precipitation of uranium. Also, the XRD pattern suggested the precipitate contains saleeite \([UO_2]_2(PO_4)_2\cdot4H_2O\) and uranyl phosphate \([UO_2(PO_3)_2]\). Further, microcosm studies could better demonstrate the potential of this bacterium for in situ application. This can strengthen the concept that bioremediation approaches could be potentially developed with *Acinetobacter* sp. USC B2 having resistance to U, which could biologically facilitate the formation of U-P for long term remediation of uranium.

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