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Isolation of urease-producing bacteria and removal effect on the Cd and Pb in filtrate

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Abstract. Heavy metal pollution in farmland soil around mining and metallurgical industrial parks in China is serious, which seriously affects crop yields and quality and threatens national public health. As an economic and environmental remediation technology, bioremediation can both reduce the metal availability in soil and decrease the accumulation of heavy metals in plants by using microorganisms. We used urea agar plates to screen and isolate urease-producing bacteria from the rhizosphere soil of agricultural crops in Cd- and Pb-contaminated farmland, and investigated its growth characteristics, the removal effects on Cd and Pb in the solution were also studied. The results showed that three urease-producing bacteria, Bacillus megateriumC6, Bacillus aryabhattaiC14, Bacillus sp.C15 were identified by screening. They had higher ability to produce urease, which were up to 876.1U/mL, 815.4U/mL and 809.3 U/mL, respectively within 24h. By studying the influence of urease-producing bacteria on the concentration of Cd and Pb in the solution, it was found that urease-producing bacteria had certain removal effect on Cd and Pb, and the removal efficiency was 51.9% and 84.1% respectively after 14 days, indicating that the urease-producing bacteria had better removal effect on Pb.

Keywords: Heavy metal pollution; Farmland soil remediation; Urease-producing bacteria;

1. Introduction

In recent years, heavy metal pollution in farmland soil in China is not optimistic, which seriously threatens the safety of crops such as food and vegetables, resulting in huge economic losses and ecological human risks.[1] The investigation shows that heavy metal pollution in farmland soil comes from industrial activities such as mining and smelting, agricultural activities such as sewage irrigation and fertilizer application.[2] China is currently the world's largest producer and consumer of non-ferrous metals, mining and smelting of nonferrous metals have become the main cause of heavy metal pollution in farmland soil, the problem of heavy metal pollution in soil around mining and metallurgical industrial parks is more prominent.[3] Therefore, the problem of heavy metal pollution in farmland around non-ferrous metal mining and metallurgy parks in China urgently needs to be solved.[4]

According to the characteristics of heavy metals, heavy metal pollution in farmland soil is characterized by concealment, accumulation, irreversibility, persistence and difficulty in treatment.[5] Currently, the methods widely used in soil heavy metal pollution control in China include physical, chemical and biotreatment. Physical restoration technology mainly includes engineering measures: new-soil method, earth exchange method, electric dynamic repair method and so on.[6] Common chemical remediation technology include chemical leaching method and chemical curing method.[7] Bioremediation is a technology that utilizes the metabolism activities of plants, animals and microorganisms to decompose and transform toxic substances, so as to repair the polluted environment. At present, the research on bioremediation technology mainly focuses on three aspects: phytoremediation, microbial remediation and plant-microbial joint remediation.[8] Compared with the other kind methods, which has the disadvantages of high cost, large

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environmental disturbance, easy introduction of secondary pollution and long phytoremediation period, the microbial remediation method has gradually become a research hotspot of heavy metal remediation in farmland soil due to its advantages of low cost, ecological friendliness and good effect.[9]

Urease-producing bacteria are widely distributed in soil and water in nature. Urease can be produced in the process of metabolism by decomposing urea to produce carbonate and NH4+, which increases the pH in the environment and is conducive to the formation and solidification of heavy metal ions. Studies have shown that urease bacteria in soil can fix more than 88% of Pb2+, Zn2+, Cd2+ and Cu2+ in 2 days.[10]

2. Materials and Methods

2.1 Isolation and Identification of Urease-producing Bacteria

The soil used for screening bacteria was collected from heavy metal contaminated farmland soil around a non-ferrous mining and metallurgy park in Hunan Province, and the rhizosphere soil of sweet potato, bean, pepper and other plants were respectively collected for screening urease producing microorganisms. The collected soil pH value was 7.03, Cd content was 30.7mg/kg, Pb content was 556mg/kg. 10g soil sample was put into 90mL sterile water and shaken in a shaking table at 25℃ and 180 r·min-1 for 2h, then stood still for 5 min. 1 mL of the upper suspension was added into 9mL sterile water, shaken evenly, and diluted to 10-7 successively by gradient. Diluent 10-4, 10-5 and 10-6 were respectively coated on urea agar medium (Table 1) and cultured in a constant temperature incubator at 25 ℃ for 3 days. Colony growth was observed and dominant strains with fast growth rate, large growth amount and obvious enzymatic reaction were selected on enrichment medium for purification. The purified strains were preliminarily identified by gram staining and colony characteristics observation, and identified by 16S rRNA sequence.

Table 1. Composition of urease screening agar plates(g/L)

Composition	Peptone	NaCl	KH_2PO_4	Urea	Glucose	Phenol red	Agar
Concentration	1	5	2	22	5	0.015	20

2.2 Determination of Growth Characteristics of Urease-producing Bacteria

The purified urease producing bacteria C6, C14 and C15 were cultured in LB medium (1g tryptone, 1g NaCl, 0.5g yeast extract, 100mL aqueous solution). Samples were taken every 4h to determine the concentration of the bacteria, which was characterized by OD600 value. the urease activity was determinated at the same time.

2.3 Determination of Urease Activity

Three urease-producing bacteria were obtained from the plant rhizosphere soils. The urease activity of these bacteria was measured according to Nessler's reagent colorimetric method. In brief, 0.1mL centrifuged supernatant of microbial fermentation broth was added to 0.9mL 3% urea solution, heated in a water bath at 35 ℃ for 7min, then added 1mL 10% trichloroacetic acid solution to terminate the reaction immediately. After the sample was slightly cooled, 1 mL Of Reagent was added and diluted with distilled water to the scale line. After 20 min of coloration, the absorbance was measured at 415 nm together with the standard curve. The concentration of NH4+ in the sample was calculated according to the standard curve, the urease activity was represented by 1 μmol free NH4+ released per minute, denoted as U.

2.4 The Effect of Urease-producing Bacteria on the Concentrations of Cd and Pb in Filtrate

The mixed culture broth of urease-producing bacteria for 24h was added to the aqueous solution containing 10mg/L Cd and 50mg/L Pb at 10% inoculation ratio. The contents of Cd and Pb in the solution and the change of pH value in the solution were sampled at 1, 3, 5, 7 and 14 days respectively

3. Results and Discussion

3.1 Isolation of Urease-producing Bacteria

Three urease-producing strains C6, C14 and C15 were isolated from vegetable rhizosphere soil by dilution spread plate method and streak plate method. Figure 1 shows that the growth situation of urease-producing bacteria cultured on urea agar medium in 30 ℃ for 7 days. NH4+ can be produced by decomposition of urea by urease secreting by Urease producing bacteria, which makes the surrounding of the medium into an alkaline state. The medium containing phenol red indicator changes from yellow to pink. The darker the color is, the stronger the urease activity is. Gram staining was performed on the three strains and observation was conducted under a microscope. It was found that the three strains were all Gram-positive bacteria, among which strains C6 and C15 were short rods and strains C14 were long rods.

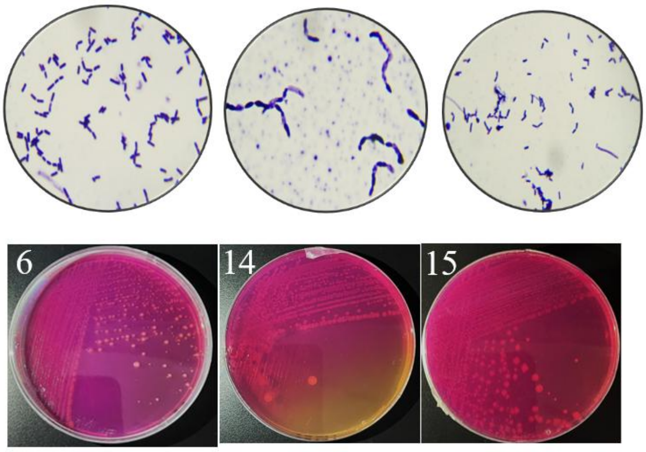
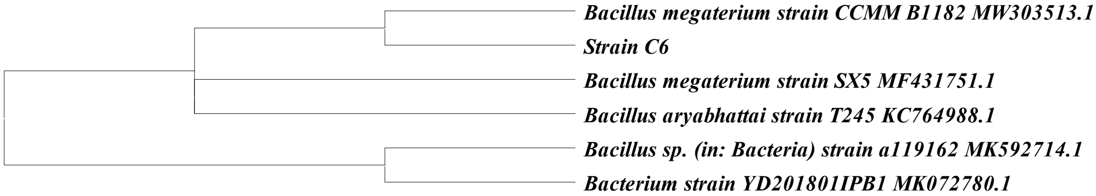


Figure 1. Morphology of strain

3.2 Sequencing and Identification of Urease-producing Bacteria

The 16S rRNA sequences of strains C6, C14 and C15 were amplified and sequenced, the homology analysis was carried out with MEGAX. As shown in Figure 2, the comparison results showed that the three strains all belonged to The genus Bacillus. Strain C6 had high homology with strain CCMM B1182 (Bacillus megaterium). Strain C14 had higher homology with strain T245(Bacillus aryabhattai). Strain C15 and strain NQS14 (Bacillus sp.) had high homology. Therefore, three kinds of target strains were obtained in this experiment, with high homology with Bacillus sp., Bacillus Megaterium and Bacillus aryabhattai, respectively.



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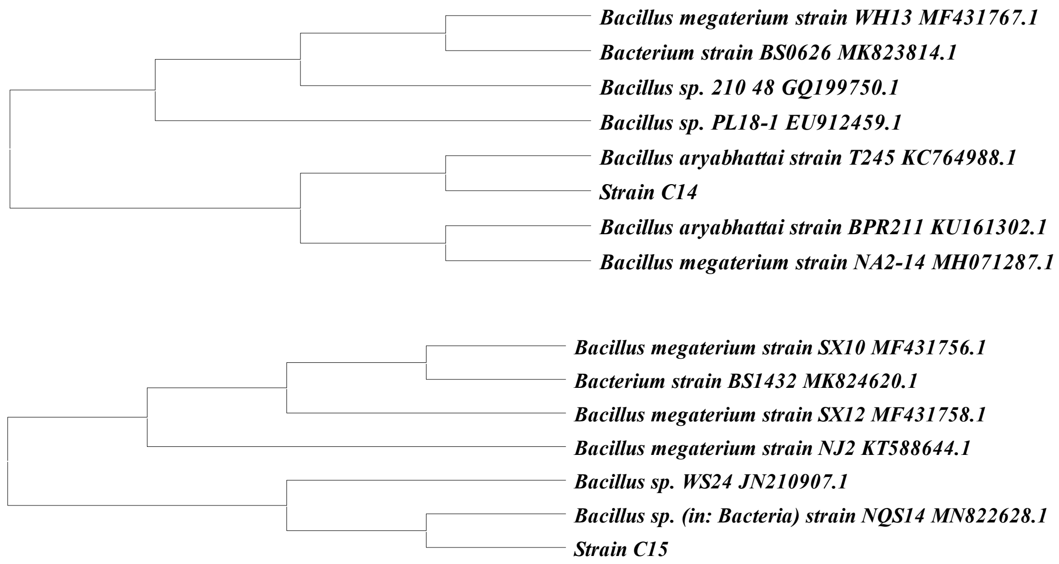

Figure 2. Phylogenetic tree of different urease-producing bacteria.

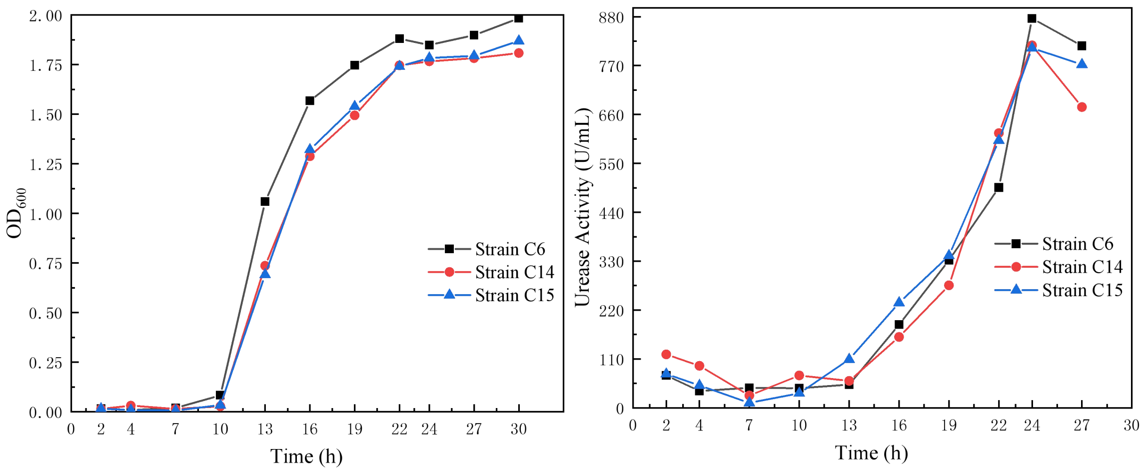

Figure 3. Growth curve and urease activity of urease bacteria during culture.

3.3 Growth Characteristics of Urease-producing Bacteria

Fig. 3 shows that the growth curves and urease activity changes of the three screened strains within 30h of culture, respectively. According to the data in the diagram, the growth period of the strains has obvious sluggish stage, logarithmic stage and stable stage. Strains C6, C14 and C15 were in the retarded stage before 10h of culture, and the microorganisms were in the adaptation stage at the initial stage after inoculation into the culture medium, with slow cell division and slow growth rate. At this time, urease activity was extremely low. After 10h, the three strains entered the logarithmic growth period, at which the metabolism of microbial cells was accelerated and the growth was rapid. The growth of strain C6 was faster than that of strain C14 and C15. The urease activity began to increase rapidly after a short reaction at 13h. At 22h, the three strains entered a stable phase, at which the rate of cell division and metabolic capacity of microorganisms decreased, the concentration of microorganisms remained stable, and the urease activity began to decrease after increasing.

3.4 The Effect of Urease-producing Bacteria on the Concentrations of Cd and Pb in Solution

Fig 4 shows the variations of Cd and Pb concentrations in the solution where strains C6, C14 and C15 were present. Through the experiment, the concentration of Cd and Pb in the control group CK

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without strain but only in the medium solution changed slightly, which may be due to the combination of the components in the medium with Cd and Pb, which reduced part of the concentration. For the repair group with three strains, the concentration of Cd and Pb decreased significantly. After 14 days of inoculation, the content of Cd and Pb decreased by 51.9% and 84%. Figure 5 shows the pH changes of the solution system during the experiment. The results showed that the pH of the CK group did not change significantly, while the pH of the BR group was 6.99 on the third day and 8.52 on the seventh day under the action of the three strains. This is due to the urease produced by the strain, which breaks down urea to produce NH4+, thus increasing the pH value of the solution.

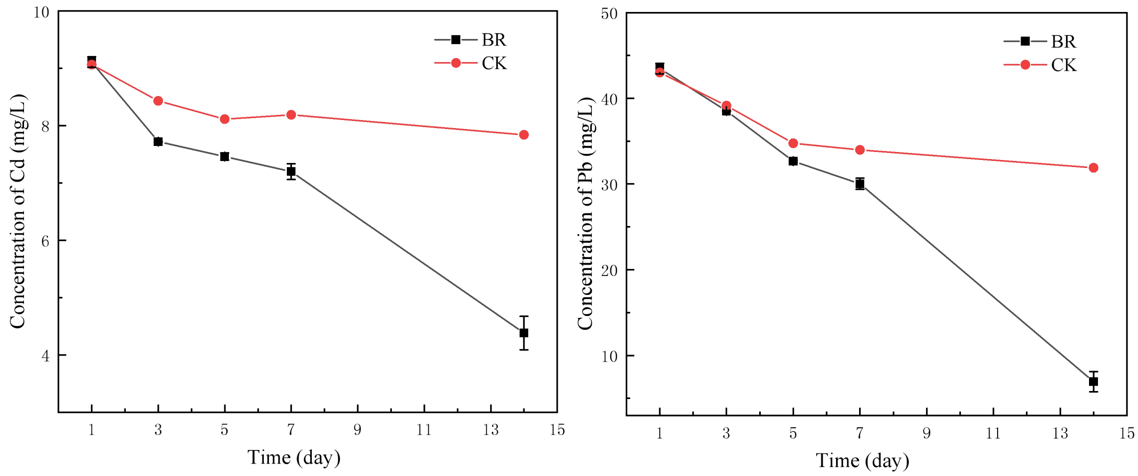

Figure 4. Effect on the concentrations of Cd and Pb in solution.

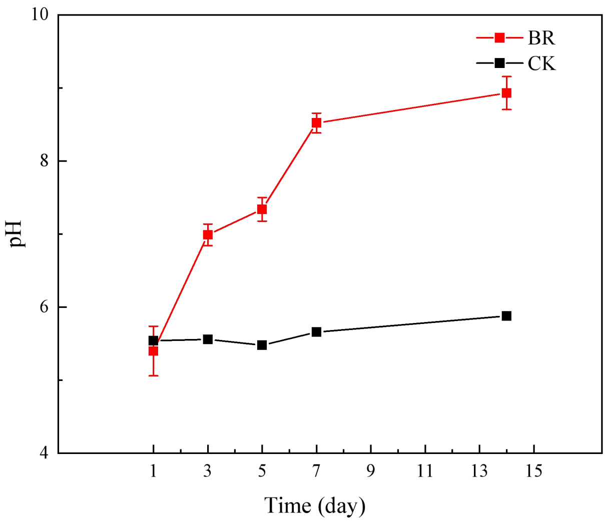

Figure 5. Trend of pH

4. Conclusion

In this research, three heavy metal-immobilizing bacteria C6, C14 and C15 with high urease production were screened and isolated from vegetable rhizosphere soil contaminated by Cd and Pb in typical mining and metallurgy parks. By gram staining, 16S rRNA gene sequencing and other methods, the target strains were Bacillus megaterium, Bacillus aryabhattai and Bacillus sp., respectively. The strain underwent slow, logarithmic and stable stages within 30h of culture. The urease activity reached the highest at 24h, which were 876.15 U/mL, 815.47 U/mL and 809.30U/mL, respectively. In aqueous solution containing Cd and Pb, the concentration of Cd and Pb can be effectively reduced. At 14 days, the removal rates of Cd and Pb are 51.9% and 84%, respectively.

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