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Original Research Paper

Adsorption Characteristics of Lead (Pb^{2+}) and Cadmium (Cd^{2+}) by an Isolated Bacterium from Soil Samples Obtained from a Tungsten Mine

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ABSTRACT

Eight bacterial strains were isolated from lead-cadmium contaminated soil samples obtained from a Tungsten mine. Pb^{2+} and Cd^{2+} adsorption abilities of the isolated strains were screened. One isolate, referred to as Strains S, was selected for further study because of its relatively high adsorption ability. Five factors affecting adsorption of Pb^{2+} and Cd^{2+} were studied, namely, the initial concentration of Pb^{2+} or Cd^{2+}, temperature, pH, biomass of Strains S, and adsorption time. Results indicated that Strains S best adsorbed Pb^{2+} when the initial concentration of Pb^{2+} was 100 mg/L, temperature was 35°C, pH was 6.5, dosage was 0.1 g, and adsorption time was 20 min. In contrast, Strain S best adsorbed Cd^{2+} at an initial Cd^{2+} concentration of 100 mg/L, temperature of 40°C, pH 6.5, dosage of 0.1 g, and adsorption time of 20 min. The experimental data were fitted to the Langmuir and Freundlich adsorption isotherm models, and the results indicated that the Langmuir model better fitted the experimental data. The results of infrared spectroscopy revealed that the functional groups involved in adsorption of Cd^{2+} and Pb^{2+} were -OH, $-NH_2$, -S=O and -P=O. Using 16S rDNA sequencing and phenotypic properties test, Strains S was identified as *Enterobacter* sp.

INTRODUCTION

Lead (Pb) and cadmium (Cd) are major metals in industrial production that are widely used in many livelihood-related industries, including electroplating, printing, dyeing and battery manufacturing. Heavy metal pollution, including Pb and Cd pollution, has received much attention from society because of the increased awareness of environmental protection. Pb and Cd have polluted vast soils, air and water. Considering that they do not decompose and are highly soluble, Pb and Cd accumulate stepwise through food chains before finally entering the human body (Dai Shugui et al. 2006). Therefore, Pb and Cd directly endanger human health and cause great damage to the environment. According to survey, the concentration of Pb and Cd in coastal water is several times to the national standard. According to a survey, the concentration of Pb and Cd in coastal water is several times to the national standard. Given the pollution situation in China, treating Pb and Cd pollution is an urgent concern.

At present, the main methods for the treatment of Pb and Cd pollution include chemical, biological and physical techniques (Jiang Xinyu et al. 2010). However, the chemical or physical methods are limited due to huge costs, operational complexity and introduction of secondary pollution. In contrast, biological methods are superior to chemical or physical methods because of their high performance, low costs, and operational simplicity (Zhou Wei et al. 2009). Thus, biological methods have received wide research attention in recent decades and are favoured by environmental protection workers (Guo Xuejun et al. 2002, Wang 2006). Adsorption of heavy metals by bacteria has been widely reported, such as adsorption of Pb and Cd by *Aspergillus aculeatus* (He Yichao et al. 2010), adsorption of up to 51 mg/g Zn by *Streptomyces ciscaucasicus* isolates (Li Huifen et al. 2010), and adsorption of up to 10 mg/g Pb by lead-resistant *Arthrobacter* isolates (Jin Yu et al. 2013). Cu-resistant *Klebsiella* was screened and tested to have an adsorption quantity of 100 mg/g (Huang Zhijun et al. 2012). Piotr Rzymski et al. (2014) investigated the adsorption of *Microcystis aeruginosa* over several heavy metals. Ding et al. (2014) studied the behaviour and mechanism in adsorption of thorium by *Streptomyces* sp. However, research on the screening and adsorption properties of *Enterobacter* sp. is lacking.

In this study, we screened the bacterial strains isolated from soils that were severely polluted by Pb and Cd near a tungsten mine. The strains were referred to as Strains S. The strains were identified through phenotypic experiments, colony characterization and 16S rDNA sequencing. In ad-

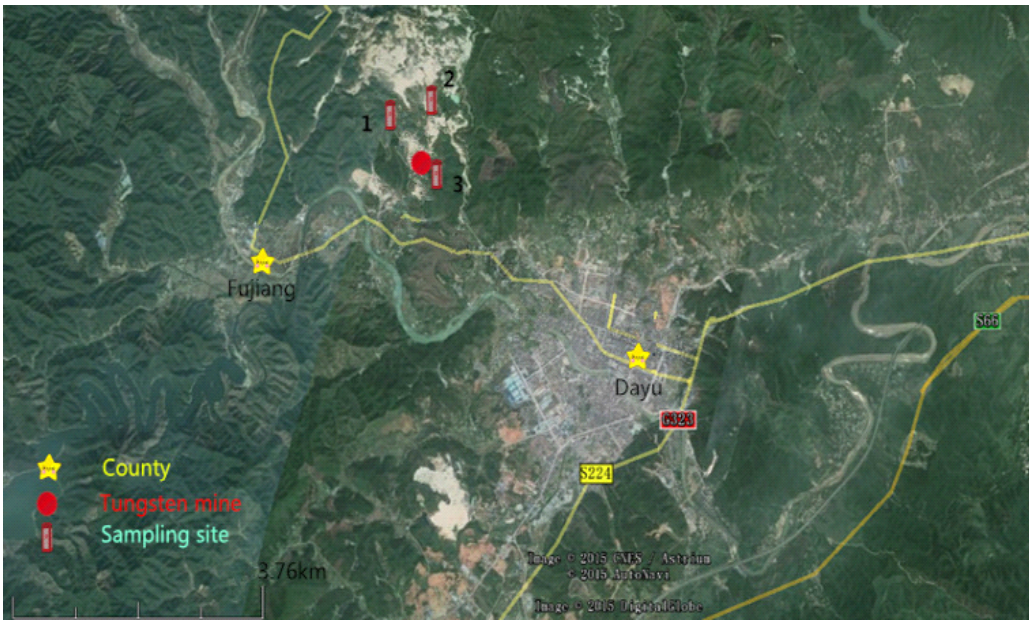

Fig. 1: The sampling geographic coordinates.

dition, we systemically studied and optimized the adsorption of Pb^{2+} and Cd^{2+} by Strains S, and preliminarily investigated the adsorption mechanism. This study will theoretically underline the future applications of bacteria in environmental remediation.

MATERIALS AND METHODS

Soil samples and culture medium: The soils for strains screening were collected from areas near a tungsten mine in Jiangxi Province, China. The soils from three sampling sites contained 967.12 mg/kg Pb and 40.22 mg/kg Cd on an average. The sampling geographic coordinates are shown in the Fig. 1.

Potato culture medium: Potatoes were peeled, cut into pieces, cooked for 30 min, and filtered through a gauze. Afterwards 60 g of the processed potatoes, 6 g of agar-agar (added during preparation of solid or semisolid medium), 300 mL of distilled water, and 6 g of glucose were mixed and sterilized under natural pH.

Selection of medium: Appropriate amounts of Pb^{2+} or Cd^{2+} to the required concentrations were added to potato medium and sterilized (Zhou Qunying et al. 2008).

Sterilization conditions: 121°C to 126°C, 30 min.

Isolation and screening of bacterial strains: Approximately 10 g of soil was added to 90 mL of sterile water. After adding an appropriate number of sterile glass beads, the mixture was oscillated for 15 min to form a suspension (Jiang Lan et al. 2008). The suspension was then diluted (10^{-2}-10^{-4}). The resulting solutions were inoculated on potato culture media containing 200 mg/L Pb^{2+} and 100 mg/L Cd^{2+}. The media were cultured in 37°C for one day, and then colonies were counted. The single colonies were inoculated separately into corresponding solid plates containing 200 mg/L Pb^{2+} and 100 mg/L Cd^{2+} for cultivation. After the colonies appeared on each plate, the characteristics of colonies were checked to determine whether these colonies were pure cultures. Microscopic examination was used to check whether the colonies were composed of a single species or not. In case of the existence of other species, the colonies should be purified until a single bacterial strain remained.

The resistance of each strain to Pb^{2+} and Cd^{2+} was then investigated. The concentrations of Pb^{2+} and Cd^{2+} in the media were increased and the growth of the colonies was observed. The strains with the highest resistance and adsorption ability were screened.

Preparation of dry strains: The selected strains were transferred to a liquid medium and cultured at 37°C under oscillation for 2 days. The liquid medium was centrifuged at 6,000 rpm for 5 min. The strains were collected, washed and resuspended in deionized water, and centrifuged again under the same conditions. The washing step was repeated three times. After the final resuspension, the strains were dried at

70°C to 80°C until constant weight was reached. The strains were then ground to powder and stored in a refrigerator at 4°C.

Single-factor adsorption experiments of dry strains: An appropriate biomass of the dry strains were weighed and added to 50 mL of water solution containing Pb^{2+} or Cd^{2+}. After oscillated adsorption as per different requirements, the solutions were centrifuged at 6000 rpm for 5 min. The concentration of Pb^{2+} or Cd^{2+} in the supernatant was then determined by atomic absorbent spectrophotometry (AAS). Experiments were performed in triplicate.

Effects of pH: Solutions with an initial Pb^{2+} or Cd^{2+} concentration of 100 mg/L were adjusted to pH 3.0, 3.5, 4, 4.5, 5.5, 6.5 or 8 using hydrochloric acid or sodium hydroxide. After addition of 0.1 g of dry strains, the solutions were oscillated at 30°C for 30 min and centrifuged, followed by measurement of the Pb^{2+} or Cd^{2+} concentration.

Effects of adsorption time: Solutions with an initial Pb^{2+} or Cd^{2+} concentration of 100 mg/L were added with 0.1 g of dry strains. The solutions were oscillated at 30°C for 5, 15, 25, 35, 45, 55, 80 or 120 min and then centrifuged, followed by measurement of the Pb^{2+} or Cd^{2+} concentration.

Effects of dosage of strains: Solutions with an initial Pb^{2+} or Cd^{2+} concentration of 100 mg/L were added with 0.02, 0.05, 0.08, 0.1, 0.12 or 0.15 g of dry strains. The solutions were oscillated at 30°C for 30 min and centrifuged, followed by measurement of the Pb^{2+} or Cd^{2+} concentration.

Effects of temperature: Solutions with an initial Pb^{2+} or Cd^{2+} concentration of 100 mg/L were added with 0.1g of dry strains. The solutions were oscillated at 15, 20, 25, 30, 35, 40 or 45°C for 30 min and centrifuged, followed by measurement of the Pb^{2+} or Cd^{2+} concentration.

Effects of initial concentration: Solutions with an initial Pb^{2+} or Cd^{2+} concentrations of 25, 50, 75, 100, 125, 150 or 175 mg/L, were added with 0.1 g of dry strains. The solutions were oscillated at 30°C for 30 min and centrifuged, followed by measurement of Pb^{2+} or Cd^{2+} concentration.

Fitting with isothermal adsorption: Solutions containing 25, 50, 75, 100, 125, 150 or 175 mg/L Pb^{2+} or Cd^{2+} were prepared and adjusted to pH 6.5. Each solution was then added with 2 g/L dry strains. The solutions were oscillated at 35°C or 40°C for 30 min and centrifuged at 6,000 rpm for 5 min. Afterwards, the concentration of Pb^{2+} or Cd^{2+} in the supernatant was determined by AAS.

Analysis of adsorptive functional groups: A mixed solution containing 100 mg/L of both Pb^{2+} and Cd^{2+} was prepared and then added with 2 g/L dry strains. The solutions were oscillated at 30°C for 30 min. The cells were centrifuged, collected, dried and used for infrared spectral

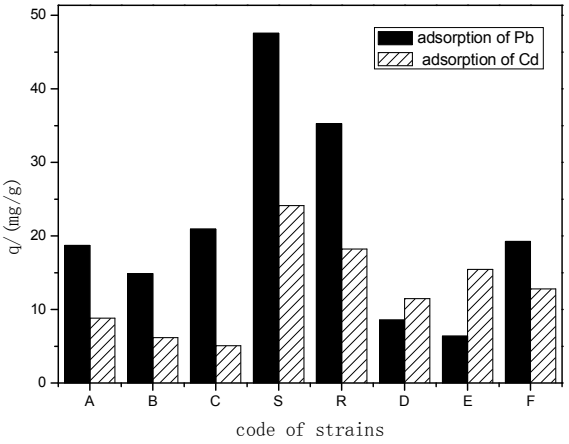

Fig. 2: Adsorption of Pb^{2+} and Cd^{2+} by isolates.

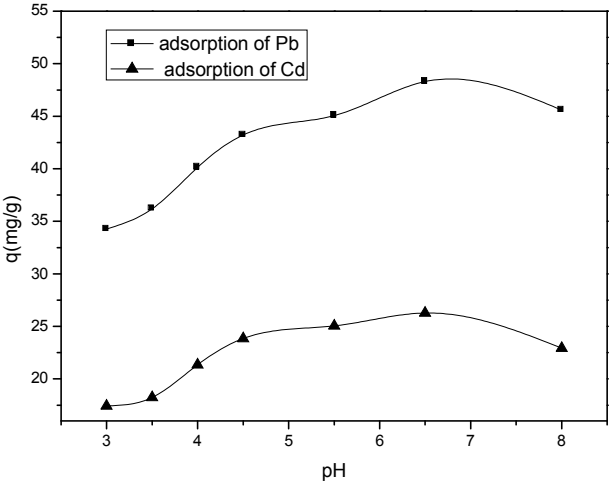

Fig. 3: Effect of pH on the adsorption of Pb^{2+} and Cd^{2+}.

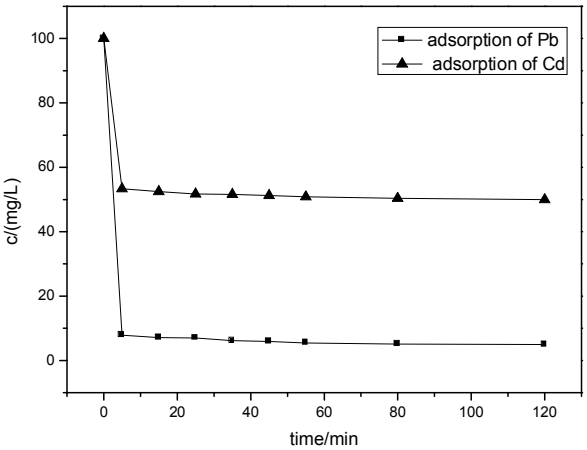

Fig. 4: Effect of adsorption time on the adsorption of Pb^{2+} or Cd^{2+}.

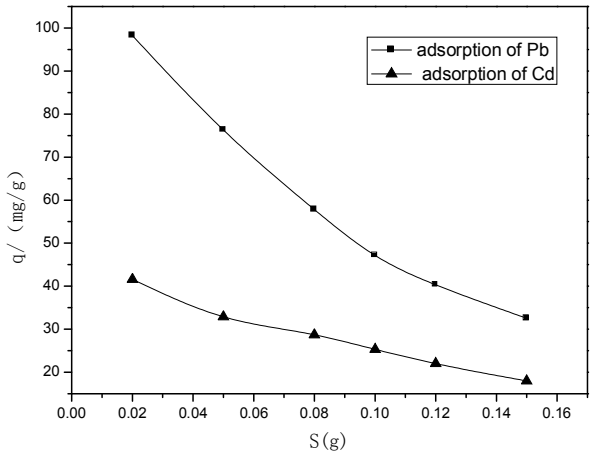

Fig. 5: Effect of biomass on the adsorption of Pb^{2+} and Cd^{2+}.

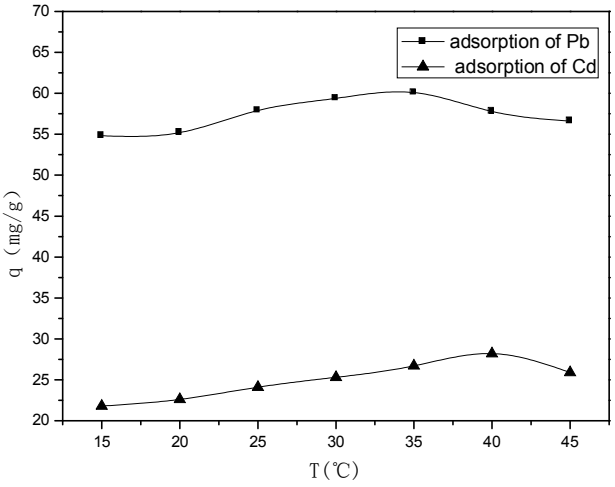

Fig. 6: Effect of temperature on the adsorption of Pb^{2+} and Cd^{2+}.

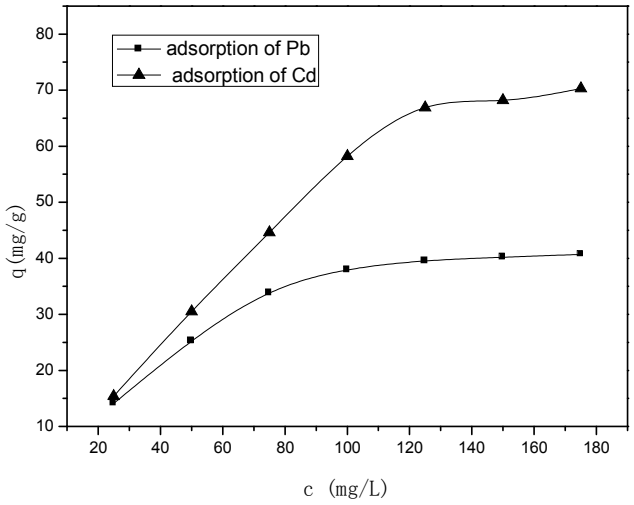

Fig. 7: Effect of initial concentration on the adsorption of Pb^{2+} and Cd^{2+}.

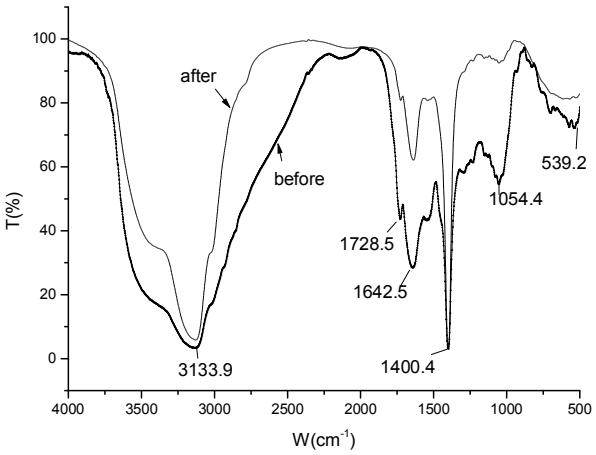

Fig. 8: FTIR spectra before and after biosorption.

analysis.

Identification of Bacterial Strains

Morphological characteristics: The isolates were lineated and removed from fresh potato media for culture, followed by observation of colony and cell morphologies.

Phenotypic analysis: The isolates were sent into methyl red, Voges-Proskauer, citrate and catalase tests according to Manual for identification of common bacteria (Dong Xiuzhu et al. 2001).

16S rDNA sequencing: Total DNA extraction, PCR amplification and sequencing were performed at Southwest Forestry University. The sequences identified were compared with those in GenBank. An unrooted phylogenetic tree for Strains S was constructed based on the genetic relationship between this strain and existing strains.

RESULTS AND DISCUSSION

Screening for bacterial strains with high adsorption of Pb^{2+} and Cd^{2+}: Eight strains were isolated from soil samples obtained from tungsten mine. Fig. 2 shows that they were generally more capable of adsorbing Pb^{2+} than Cd^{2+}. The strains most effective in adsorbing Pb^{2+} were strains S and R, adsorbing quantities up to 47.4 and 39.3 mg/g, respectively. The strains most effective in adsorbing Cd^{2+} were also strains S and R, adsorbing quantities up to 24.1 and 18.2 mg/g, respectively. Based on these results, Strain S had the highest adsorption ability for both Pb^{2+} and Cd^{2+}, so it was selected for use in further experiments.

Effect of pH on adsorption ability: As shown in Fig. 3, the quantity and rate of adsorption of Pb^{2+} or Cd^{2+} by Strain S, both increased with rising pH and then peaked at pH 6.5.

The adsorption quantities of Pb^{2+} and Cd^{2+} were 48.3 and 26.3 mg/g, respectively, and the removal rates were 96.6% and 52.6%, respectively.

Very low pH is unfavourable for the adsorption of Pb^{2+} or Cd^{2+}, because abundant free hydronium ions (H_3O^+) present at low pH bind with the functional groups (e.g. hydroxy and amido) on the surface of the strains (Qin Yuchun et al. 2008). Thus, H_3O^+ competes with Pb^{2+} or Cd^{2+} and inhibits the strains from adsorbing Pb^{2+} or Cd^{2+}, leading to lower pH and further complicating the adsorption of Pb^{2+} or Cd^{2+}. With the increase in pH, the inhibitory action of H_3O^+ on Pb^{2+} and Cd^{2+} is weakened. However, after the peak adsorption at pH 6.5, further increase in pH precipitates Pb^{2+} and Cd^{2+} through binding with the OH^- that gradually appears. Moreover, increasing pH increases the negative charge on the surface of the strains. Thus, the surface of the strains is covered with metal hydroxides, which are unfavourable for the exposure of surface groups in the strains. These two factors cause the abrupt decline in the adsorption quantity and rate under high pH. These results are consistent with the findings of two previous reports (Fan et al. 2008).

Effects of incubation time on adsorption ability: As shown in Fig. 4, the trends in adsorption of Pb^{2+} and Cd^{2+} by Strain S were very similar. Within the first 5 min, the concentrations of Pb^{2+} and Cd^{2+} declined sharply by 91.7% and 46.7%, respectively, after which the concentrations remained constant. Nevertheless, the overall trend was adsorption, probably because the initial concentration of Pb^{2+} or Cd^{2+} was too low. After rapid adsorption at the early stage, the density of adsorption sites on the surface of the strains significantly decreased, leading to the significantly reduced probability of collision between ions and the adsorption sites. Fig. 4 also shows that the adsorption rate and ability of the strains, both changed with the type of heavy metal, indicating that the properties of these heavy metals differed to some degree.

Effect of biomass on absorption ability: As shown in Fig. 5, the adsorption rate of both Pb^{2+} and Cd^{2+} increased with the increase in strain biomass, but the adsorption quantity per unit of Strains S biomass gradually decreased. At a strain biomass of 0.1 g, the adsorption rates of both Pb^{2+} and Cd^{2+} stabilized. These changes were due to the following possible reasons: After addition of the strains, the numbers of corresponding adsorption sites for Pb^{2+} and Cd^{2+} gradually increase, but the concentrations of Pb^{2+} and Cd^{2+} did not change. Thus, the removal rates of metal ions stably increased, but the adsorption quantity per unit of strain biomass gradually declined. Nevertheless, these results indicated that if the sites on strains surface are not saturated, the strains will further adsorb Pb^{2+} or Cd^{2+}; thus, further addition of strains is not necessarily beneficial. Thus, in real production, the strain biomass should be well regulated and weighed by considering both the adsorption rate and adsorption quantity to ensure full use of adsorption sites.

Effects of temperature on adsorption ability: As shown in Fig. 6, too low or too high temperatures were unfavourable for adsorbing Pb^{2+} or Cd^{2+}. At 15°C, the adsorption of Pb^{2+} and Cd^{2+} was not ideal. With further increase in temperature, the adsorption rate and quantity both increased. The adsorption quantity of Pb^{2+} peaked at 35°C with a value of 60.1 mg/g, whereas the adsorption of Cd^{2+} peaked at 40°C with a value of 28.2 mg/g. However, the adsorption quantities of Pb^{2+} and Cd^{2+} decreased at different degrees with further increase in temperature. Fig. 6 also shows that the adsorption of Cd^{2+} by Strain S was more temperature-dependent compared with the adsorption of Pb^{2+}. Moreover, the increase in temperature within a certain range, helped improve the adsorption efficiency. A possible reason is that, lower temperature reduced chemical adsorption, leading to lower adsorption efficiency by the strains. However, the increase in temperature led to enhanced chemical adsorption and a gradual improvement in the adsorption efficiency. However, extremely high temperatures resulted in deformation of adsorption sites on cell surfaces, leading to lower adsorption efficiency of metal ions (Xu Aiqing et al. 2013). Moreover, the strains partially desorbed the metal ions after adsorption. As a result, the adsorption effect was first enhanced, reached its maximum and ultimately weakened.

Effects of initial concentration on adsorption ability: As shown in Fig. 7, the adsorption quantity per unit strain biomass increased with increasing initial concentrations of Pb^{2+} or Cd^{2+}, probably because higher concentrations correspond to a higher driving effect (Ho et al. 2000). However, the adsorption quantity of Pb^{2+} or Cd^{2+} per unit of strain biomass gradually stabilized, and no further increase was observed with increasing initial concentration. A possible reason is that the adsorption sites of Pb^{2+} or Cd^{2+} on the surface were saturated, so the adsorption and desorption rates were balanced. Moreover, higher concentrations of Pb^{2+} or Cd^{2+} destroyed the strains' structure. At low concentrations, the majority of adsorption sites were involved in adsorption, but at high concentration, only some of the sites were involved. Thus, these two causes led to the stabilization of adsorption.

Fitting of adsorption isotherm: Bacterial adsorption of heavy metals can be quantified by an adsorption isotherm model. The adsorptions of Pb^{2+} and Cd^{2+} by Strain S were fitted by Langmuir and Freundlich isothermal adsorption models. The results are listed in Table 1 (R^2 is the correla-

Table 1: Langmuir and Freundlich model constants.

Metal ion	Langmuir model			Freundlich model		
	q_{max}	b	R^2	K_F	$1/n$	R^2
Pb^{2+}	65.0195	0.01538	0.99760	1.9929	0.29950	0.99357
Cd^{2+}	41.1015	0.02433	0.99946	9.7252	0.37157	0.99528

Note: q_{max} is in mg/g; b is in L/mg.

Table 2: Phenotypic properties of Strain S.

Index	Result	Index	Result
Glucose	+	Methyl Red	-
Lactose	+	Voges-Proskauer	+
Maltose	-	Citrate	+
Mannitol	+	H_2S	-
Sucrose	+	Urea	+
Indole	-	Motility	+
Gram staining	-	Catalase	+

Note: "+" indicates positive test; "-" indicates negative test.

tion coefficient measuring the goodness-of-fit between the adsorption of Pb^{2+} or Cd^{2+} and the models).

As depicted in Table 1, Pb^{2+} adsorption highly fit the Langmuir and Freundlich models, with $R^2 = 0.9976$ and 0.99357, respectively. However, q_{max} obtained from the Langmuir model representing the adsorption quantity of Pb^{2+} was closer to the maximum adsorption quantity obtained from the experiment. Thus, Pb^{2+} adsorption by Strains S fit the Langmuir model better, indicating that the adsorption process was dominated by monolayer adsorption. Cd^{2+} adsorption highly fit both Langmuir and Freundlich models also, with $R^2 = 0.99946$ and 0.99528, respectively. Similar to Pb^{2+} adsorption, q_{max} obtained from the Langmuir model was closer to the maximum adsorption quantity, indicating a better fit than the Freundlich model. Thus, the adsorption of both Pb^{2+} and Cd^{2+} by Strains S was dominated by monolayer adsorption to some degree. These results also indicated that the adsorption by the strains was a complex process, limiting the application of the Freundlich model.

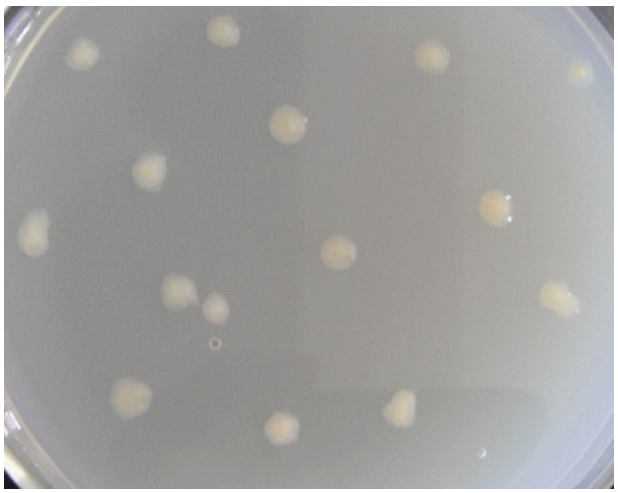

Fig. 9: Colonies of Strains S.

Analysis of adsorptive functional groups: As shown in Fig. 8, clear absorption peaks appeared in the whole wave range. Based on the relevant literature (Lu Yongquan et al. 1985), the strong and broad peak at 3133.9 cm^{-1} was ascribed to the stretching vibrations of –OH and –NH; the peaks at 1728.5 and 1642.5 cm^{-1} were ascribed to the stretching vibration of –C=O in –CO–NH; the peak at 1400.4 cm^{-1} was ascribed to the asymmetric bending vibrations of $–CH_3$ and $–CH_2$; the peak at 1054.4 cm^{-1} was ascribed to the stretching vibrations of S=O, P=O, and P–O; the peak at 533.3 cm^{-1} was ascribed to the stretching vibrations of C–Cl, C–Br, and C–I.

The infrared spectra before and after adsorption showed that the absorption peaks at 3133.9, 1728.5, 1642.5, 1400.4, and 1054.4 cm^{-1} all changed after adsorption. Thus, functional groups, namely, –OH, $–NH_2$, –S=O, and –P=O play key roles in the adsorption of Pb^{2+} and Cd^{2+}.

Identification of bacterial strains: The cells of Strain S are short, rod-shaped, and flagellated without spores. On potato glucose solid media, the colonies are morphologically characterized by wet surfaces, smooth margins, stickiness, white colour and non-diffusion. The colonies are shown in Fig. 9.

The biochemical indices of the strains are listed in Table 2. Strain S is Gram-negative, motile, and able to ferment four sugar alcohols, namely, glucose, lactose, mannitol and sucrose. It produces various acids and gases, but cannot ferment maltose. It is positive in the catalase test, Voges-Proskauer test, citrate utilization test and urea test, but negative in indole test, methyl red test and H_2S test.

The 16S rDNA base sequences of this strains were sent to Blast matching with data on GenBank. Several strains, whose base sequences were homogenous with Strain S were identified. Based on the homology degree of Strain S with these strains, a phylogenetic tree of Strain S was constructed to determine its classification. The phylogenetic tree is illustrated in Fig. 10. This strain was identified to be *Enterobacter* sp.

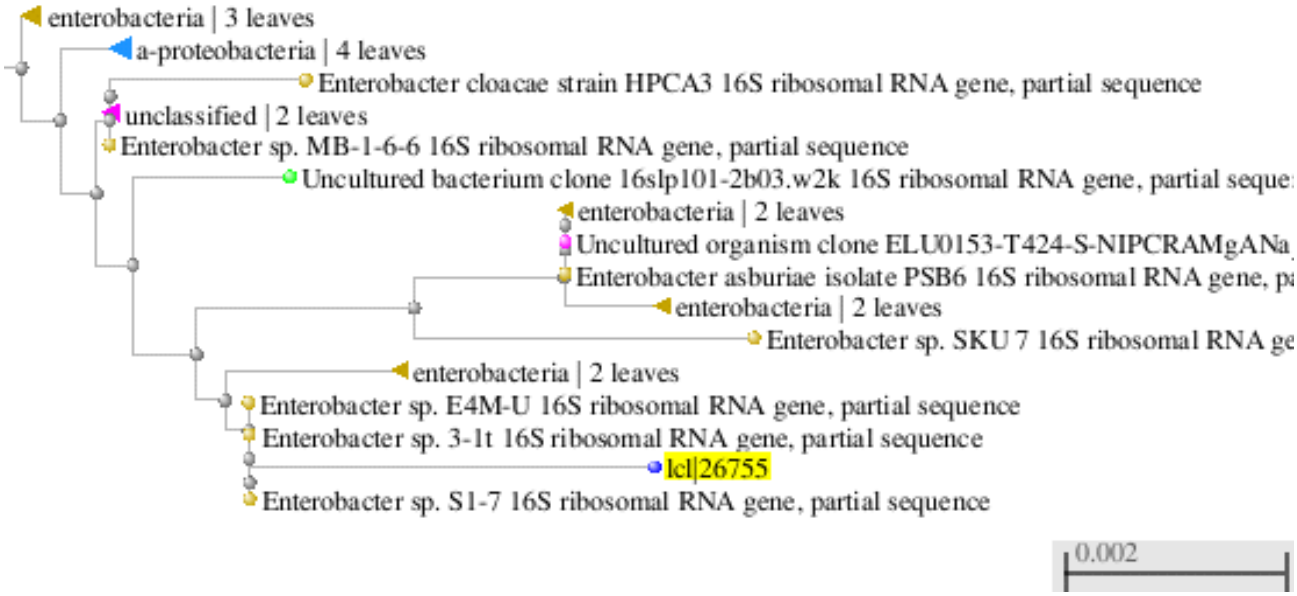

Fig. 10: Phylogenetic tree based on the similarity of 16S rDNA sequences of Strains S to other type strains.

CONCLUSIONS

1. From farmland soils with severe Pb and Cd pollution near a tungsten mine, one high-performance strain capable of adsorbing Pb^{2+} and Cd^{2+} referred to as Strain S in this paper was screened out and identified to be *Enterobacter* sp.
2. Strain S rapidly adsorbed Pb^{2+} and Cd^{2+}, and the maximum adsorption quantities within 5 min were both greater than 90%. When the strains biomass was greater than 0.1 g, the abilities of this strain over Pb^{2+} and Cd^{2+} both stabilized, but with further addition of dry strains, the removal ratios of Pb^{2+} and Cd^{2+} were unchanged. At pH 6.5, the adsorption quantities of Pb^{2+} and Cd^{2+} were up to 48.3 and 26.3 mg/g, respectively. When the initial concentration of Pb^{2+} or Cd^{2+} was 100 mg/L, the adsorption effect was at maximum. The optimal adsorptive temperatures over Pb^{2+} and Cd^{2+} were 35°C and 40°C, respectively.
3. The Pb^{2+} and Cd^{2+} adsorption of this strain fits both the Freundlich and Langmuir models, but fits the later model better. Functional groups, including –OH, $–NH_2$, –S=O, and –P=O, play key roles in the adsorption process.

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