academicJournals

Vol. 8(8), pp. 476-481, August 2014
DOI: 10.5897/AJEST2013.1640
Article Number: 368E1CC46965
ISSN 1996-0786

http://www.academicjournals.org/AJEST

African Journal of Environmental Science and Technology

Full Length Research Paper

The absorption and scavenging ability of a bacillus in heavy metal contaminated soils (Pb, Zn and Cr)

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Received 26 December, 2013; Accepted 25 July, 2014

A bacterial strain, which was able survive Pb, Zn and Cr -heavy metal compound culture was isolated from the soils of Shanjia Villiage, Qufu City, Shandong Province. An observation of the morphological and biochemical characteristics of the compound and the analysis of 16SrDNA sequence revealed that it was *Bacillus*. Standing liquid culture was used to study the tolerance of Pb Zn Cr -heavy metals compound and the ability to absorb and clean the heavy metal compound. Further study was made to compare the absorbing ability of Cu, Cd and Hg.

Key words: *Bacillus*, heavy metal, Pb, Zn, Cr.

INTRODUCTION

With the rapid development of economy and society, the exploitation of coal resources in China increases gradually and environmental problems caused by coal gangue have become increasingly serious (Shao and Cao, 2002). Coal gangue is the main component of coal slag. According to statistics, the cumulative stacked gangue is more than 40 tons (Guo, 2007), but the annual growth rate is still 1-2 million tons. There are heavy metals such as Cr, Pb, Zn, Cd, Cu and other heavy metal elements in the gangue. When there is accumulation of harmful heavy metals in the soil to a certain degree, it will have toxic effects on soil - plant system, which not only causes soil degradation, decline in crop yields and quality, but also pollutes the surface water and groundwater through runoff and leaching, deterioration of water environment, and direct contact with food chain and other ways to endanger human health and life. In the toxic heavy metals, pollution in soil ecological system is long-term and irreversible (Liu, 2008); but the problem is not obvious. Therefore, soil ecosystem metal, heavy metal pollution and prevention especially in the international arena have been a difficult and hot research topic (Chi, 2006).

Microbial remediation is the use of microorganisms (bacteria, fungi, indigenous alien gene engineering bacteria) on the metabolism and transformation of pollutants, degradation of pollutants, mainly used for degradation of organic pollutants in soil. Microbial remediation technology does not destroy the soil environment for plant growth, does not form secondary pollution and does not transfer the pollutants to residual

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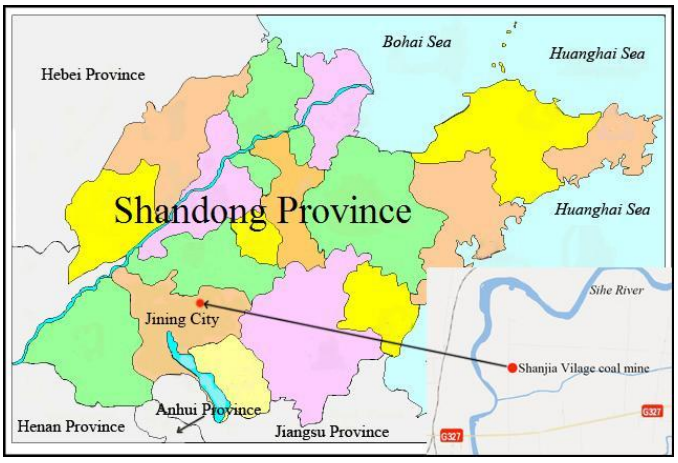

Figure 1. The location map of sampling point.

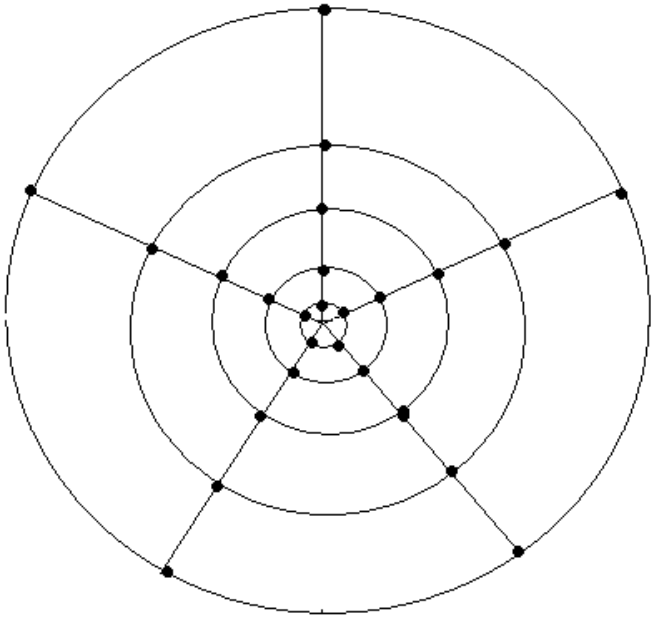

Figure 2. Soil sampling distribution.

Problems (Zhang and Xia, 2000). It can maximize the removal of pollutants or make contaminants harmless. It interferes less in surrounding environment and has the advantages of good environmental and social benefits (Chen et al., 1997). Bioremediation technology, the rapid rise in recent years, has become a green field of scientific research (Ebbs, 1997; Weon, 2011).

At present, domestic and overseas scholars are launching a lot of research on the coal mine area and soil heavy metal management (Li, 2009; Liu, 2009; Murzaeva, 2004). Domestic and foreign experts have adopted the toxic improver method, using local traditional and chemical methods to solve the problem of heavy metal contamination in soil. However, because of their own limitations, they have failed to get the ideal method for soil heavy metal management (Tang, 1996; Wang, 1996). In recent years, there are a many researches about heavy metal resistant bacteria, which are mainly concentrated in heavy metals such as Cd, Cu and As. Therefore, the composite metal reports is even less.

This work studied the soil polluted by coal gangue, based on the theory of microbial remediation technology. Isolated strains can fight a variety of heavy metals. This work at the same time fills the gaps in the domestic research of heavy metal pollution in China caused by coal gangue, and shows how to prevent the pollution of the gangue based on background and scientific information.

This test on coal gangue from mountain soil separated strains that have the ability to absorb compositing metals-Pb, Zn and Cr. After the physiological and biochemical reaction of the strains was identified as well as 16SrDNA, an auxiliary research was done for the bacteria to explore their resistance to Cu, Cd and Hg and their ability to remove degradation.

MATERIALS AND METHODS

The source of the bacteria

The experiment soil samples are taken from Shanjia Vilage Coal Mine in Qufu City (Figure 1), Shandong Province.

The distribution point of the samples is shown in Figure 2; with distance of 2, 5, 10, 15, 20 and 25 m. After retrieving six samples, they were mixed fully and set aside for pretreatment, with quartiles.

The screening culture of the strains

The preparation of the soil solution

The mixed sample was collected into laboratory cultures domesticated for seven days. 1 g of the mixed sample was used, which was diluted with 99 mL of sterile water. After, it was oscillated for 20 min, and stood still for 20 min. The upper clear liquid of 0.5 mL was taken. 4.5 mL sterile water was added into a test tube and the soil diluents concentration of 10^{-3} was obtained. In accordance with the above method, one can get diluted soil concentration gradient of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.

The preparation of the culture media

Beef extract-peptone medium: 5 g beef extract; 10 g peptone; 5 g NaCl; 15-20 g agar; 1000 mL H_2O; pH 7.2-7.4. Potato medium: 200 g selected high quality potatoes; 20 g glucose; 15-20 g agar; 1000 mL H_2O; natural pH. GAO 1st synthetic culture medium: 20 g soluble starch; 1 g KNO_3; 0.5 g $K_2HPO_4{\cdot}3H_2O$; 0.5 g NaCl; 0.5 g $MgSO_4{\cdot}7H_2O$; 15-20 g agar; 0.01 g $FeSO_4$; pH 7.2-7.4.

The culture medium components were configured for preparation and the autoclave was used for sterilization for 20 min under 121°C. After the medium was cooled to about 50°C, 60 mg Pb $(NO_3)_2$, 50 mg $ZnSO_4$ and 80 mg $K_2Cr_2O_7$ were added (every three parts separately) to the medium. It was oscillated and shaken to have a dissolved mix. All the operations were done on bench top.

Table 1. The specific microorganisms culture conditions.

Temperature (°C)	Microbiota	Culture medium	Soil solution concentration	Incubation time
37	Germ	Beef extract-peptone medium contains Pb, Zn and Cr	10^{-5}, 10^{-6}, 10^{-7}	24 h
28	Fungus	Potato medium contains Pb, Zn and Cr	10^{-5}, 10^{-6}, 10^{-7}	48 h
28	Actinomycetes	GAO 1th synthetic culture medium contains Pb, Zn and Cr	10^{-4}, 10^{-5}, 10^{-6}	5 day

The screening of the strains

The strains were kept in 9 cm plate for development, using the pouring plate method. The specific culture conditions are in Table 1. Each concentration has three parallel groups, and the growth of the strains was recorded.

Domestication of the strains

Good growth was obtained by training the resistant strains colony with a cross, using the methods of purification. Purification of culture medium and training methods are used to screen the same species. All strains were purified for three times. At last, the individual colony was purified. All the strains were kept in the refrigerator (4°C) for preservation.

The study of the ability of the strains for removing degradation

Heavy metal processing method

The medium component is 1.2.2, but agar was not added. The four concentrations were done at the same time. The concentrations of the bacteria are screened as a benchmark concentration. The concentrations were set ordinarily for five, ten and twenty times and vaccination training was done. The liquid stalling training method was taken for study. The bacteria culture conditions are given in Table 1; the growing strains were not added to the sune-vector.

Research methods

The liquid static method was used for training; and the liquid static training of the bacteria cultures is centrifuged by high-speed freezer in 13000 r/min and 4°C. The concentration of heavy metal was measured from the centrifugal machine supernatant by the atomic absorption photometer.

Identification of the strains

The bacteria strains with the best degradation ability were selected for identification.

Morphological observation of strains

The morphology of the bacteria colony in the beef was observed specially in a flat plate. Mycelial morphology was observed under oil lens by using gram staining, and the stained spore was added for observation.

Physiological and biochemical reactions of the strains

Glucose oxidation fermentation test, oxidase test, starch hydrolysis test, Acetyl methyl methanol test, methyl red (MR) test, catalase test, producing H_2S test, indole test, nitrate reduction test, and so on were done for the selected bacterium.

PCR amplification and sequence analysis of 16SrDNA

16SrDNA sequence analysis : total DNA strain extracted was cultured , as a template, using primers 8f 5'AGAGTTTGATCCTGGCTCAG 3 '20 bp and 1492r 5'GGTTACCTTGTTACGACTT 3 '19 bp; the PCR product was cloned by the Shanghai Biological Engineering Co., Ltd. for purification identification.

Resistance of the strains to Cu, Cd and Hg

Based on the optimal growth conditions, the screening strains can degrade Cu, Cd and Hg as well as composite metals- Pb, Zn and Cr, using the same methods. The benchmark concentration is 0.5 mg/L. To calculate its removal efficiency of Cu, Cd and Hg as compared to the initial removal efficiency experiments, more comprehensive experimental results are drawn.

RESULTS

Eight strains were obtained after screening: three bacteria, three fungi and two actinomyces. The study of the ability of degradation and removal showed that the strain with most powerful removal and degradation ability was bacteria. The strains with the highest percentage of degradation and absorption are three selected bacteria.

Strains identification

Morphological observation of strains

The selected bacteria are round, neat at the edge, ecru white, opaque, gram-positive and are found in aspen. The form is microscopically shown in Figure 3.

Physiological biochemical reactions of the strains

The results of physiological biochemical reactions of the strains are seen in Table 2.

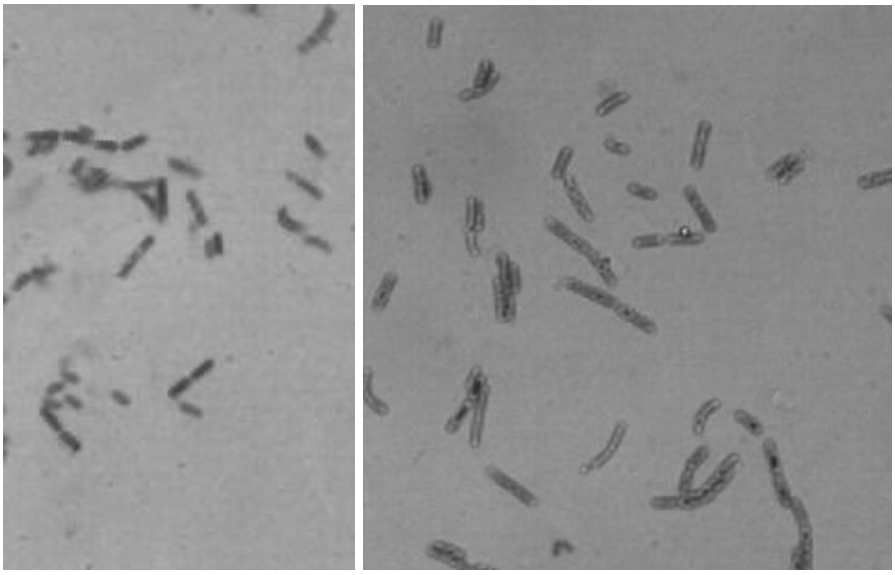

Figure 3. The form observations microscopically.

Table 2. Results of physiological and biochemical reactions of the strains.

Physiological biochemical reactions	Result
Oxidase	-
Catalase	+
Amylolysis	+
Oxidation fermentation of glucose	+
Indole test	-
Nitrate reducing reaction	+
Methyl red test (MR)	+
Acetyl methyl methanol test	+
Produce H_2S test	-

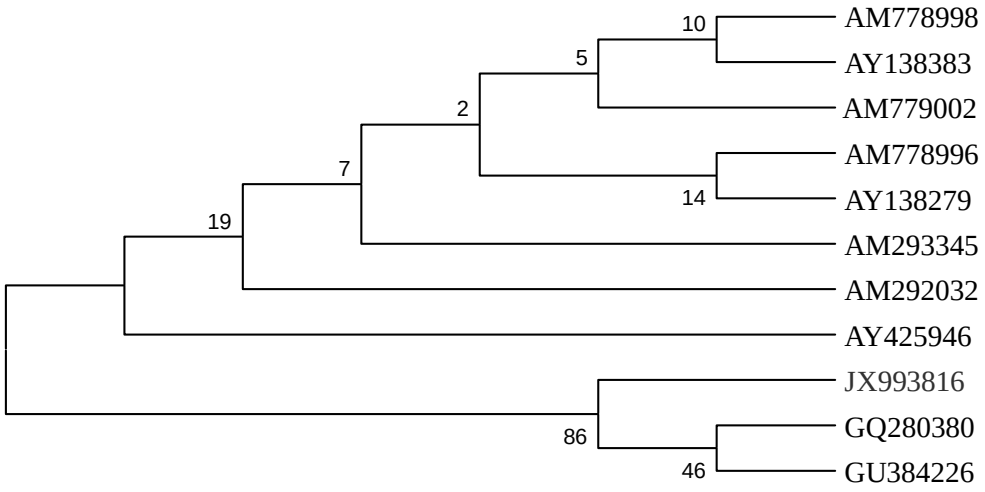

Figure 4. Evolutionary tree.

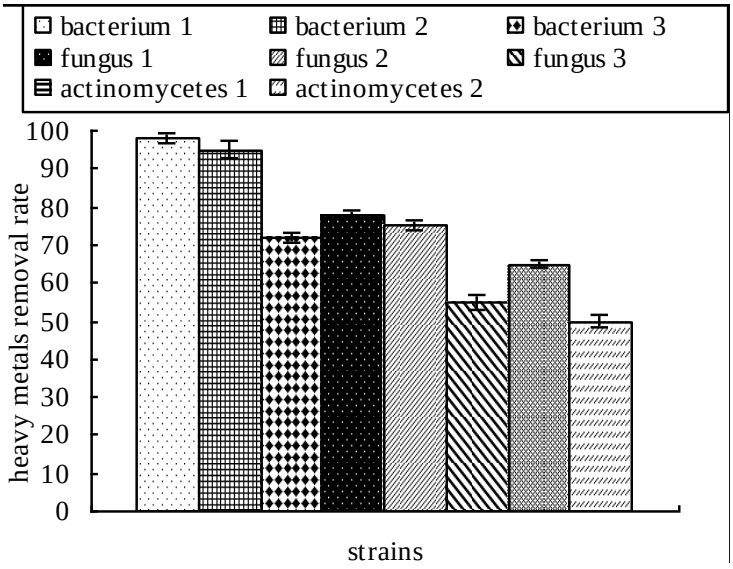

Figure 5. Different rates of strains for heavy metals removal. Annotations: This compound metal concentration is of the benchmark concentration (namely 1 times concentration).

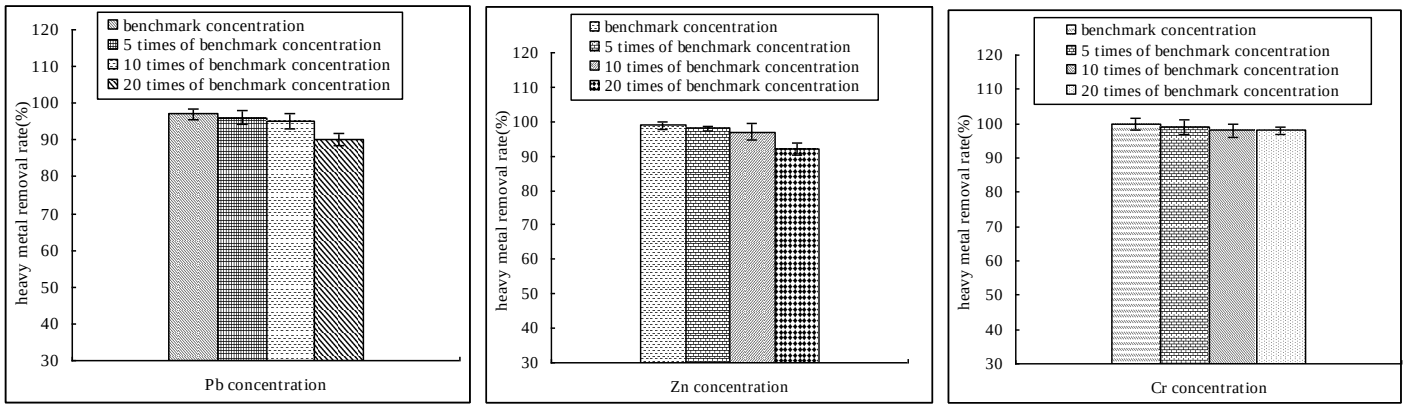

Figure 6. The strain away rate of Pb, Zn and Cr.

Sequence analysis of 16SrDNA

16SrDNA sequence specific to the Shanghai SANGON Biological Engineering Technology Service Co. Ltd selected strain is 1640 bp (GenBank accession number JX993816). BLAST searches for the series, using Clustal W and PHYLIP software to analyze and build the phylogenetic tree (Figure 4).

From the results of the analysis, selected strain and Bacillus homology was 100%. Generally, 16S rDNA sequence homology of less than 98% can be considered to belong to different types; homology less than 93~95% can be considered to belong to different genera (Devereux et al., 1990; Fry et al., 1991). Thus, combining the selected strains based on their morphological, physiological and biochemical reaction characteristics, *Bacillus cereus* is identified.

The ability to degrade and remove Pb, Zn and Cr

The analysis of the ability to degrade and remove different strains of composite metals- Pb, Zn and Cr

Figure 5 shows clearly: (1) Different microorganisms have different ability to degrade and remove heavy metals; the overall performance is: *Actinomyces* > *fungi* > *bacteria*. (2) Different strains (bacterium 1, 2, 3) of the same kind of microorganism (such as bacteria) have different degradation and removal ability.

The ability to degrade and remove heavy metal compound

In Figure 6, the separated bacilli all have certain resistance to Cu, Cd and Hg, and have certain Degrada-

tion removal ability. With the increase of metal concentration, the removal rate drops down. From the figure, we can also see that the *bacilli* remove three kinds of metal in the benchmark concentration.

DISCUSSION

In this work, samples are taken from coal gangue mountain soil; and the microbe's resistance to the composite metals- Pb Zn Cr is studied. Bacteria extracted from the soil solution were configured. From the experiment, we can draw the following conclusions:

1) Eight strains are obtained after screening three bacteria, three fungi and two actinomyces. After their morphological, physiological and biochemical reaction observation as well as 16 SrDNA sequence analysis, it is shown that the bacteria are gram positive bacilli. There are different types of media due to differences in nutrients for enrichment and separation of different species of microbes. It does not only improve the separation efficiency of strains and selective enhancement, but also guarantees the reliability and feasibility of the experiment. So it is the premise of the experiment to be carried out smoothly.
2) Experimental results show that different strains that degrade and eliminate heavy metals exhibit different processing capabilities. The ability to process heavy metals is strongest among bacteria, followed by fungi and actinomycetes. In addition, different strains of the same bacteria species also exhibit different processing capabilities. The results show that with increased concentration of heavy metals, their ability to remove those decreases.
3) Further research proves that bacillus also has certain ability to remove Cu Cd Hg.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

This study was supported by Shandong Provincial Natural Science Foundation (ZR2013EEQ009), China; Project of Qufu Normal University (No.XJZ200825).

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