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Full Length Research Paper

Heavy metal resistance by two bacteria strains isolated from a copper mine tailing in China

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Two highly heavy metal resistant indigenous bacterial strains, DX-T3-01 and DX-T3-03, were isolated from the biggest tailing in Asia-Dexing copper mine 4# tailing. The strain DX-T3-01 exhibited high tolerance to cadmium: 10 mM Cd^{2+} with yeast tryptophan peptone glucose (YTPG) agar plates and 18 mM in liquid medium. The strain DX-T3-03 was highly resistant to zinc and could endure 35 mM Zn^{2+} with YTPG agar plates and 40 mM in liquid medium. They also showed tolerance to other heavy metals, such as copper, lead and nickel. The morphology, physiological and biochemical characteristics of the two strains were examined by scanning electron microscope (SEM) and BIOLOG. The strains showed different metabolic patterns of carbon sources. The strain DX-T3-03 had a larger range of antibiotic resistance than DX-T3-01. On the basis of 16S rDNA sequencing, the two strains were identified as *Ralstonia pickettii* strain DX-T3-01 and *Sphingomonas* sp. strain DX-T3-03, respectively. This study supplied potential bacterial materials for tailing bioremediation in the future.

Key words: Heavy metals, tolerant bacteria, isolation, mine tailing.

INTRODUCTION

Mine tailings usually contain high concentrations of metals (Cu, Zn, Fe, Mn, Ni, Pb and Cd) ranging from 1 to 50 $g \cdot kg^{-1}$ (Monica et al., 2008). Heavy metal contamination is one of the most important environmental concerns from mine tailings. Metals are a significant toxic factor to biota in the environment. For example, heavy metals may decrease metabolic activity and diversity as well as affect the qualitative and quantitative structure of microbial communities (Giller et al., 1998). Each heavy metal has unique toxicity or function. Cadmium is widespread and one of the most toxic pollutants, it is non-essential but poisonous for plants, animals and humans. It has high toxicity even at low concentrations (Gupta and Gupta, 1998). In contrast, zinc and copper can enhance microbial growth at low concentrations but suppress growth at high concentrations (Ge et al., 2009).

For treating heavy metal contaminated tailing and soils, bioremediation is the most efficient and least costly method (Ge et al., 2009). So, recent interest in the reclamation of abandoned mine tailings focuses on revegetation or phytostabilization (Munshower, 1994). A number of mine tailing reclamation studies have emphasized a strong association between the establishment of a stable plant community and the abundance and composition of microorganisms (De La Iglesia et al., 2006; Monica et al., 2008). Some reports have also shown that indigenous microbes and plant–microbe symbionts tolerate high heavy metal concentrations in different ways and may play a significant role in the restoration of contaminated soil (Carrasco et al., 2005; Ge et al., 2009). It is important to study the indigenous microorganisms in heavy metal polluted sites. It may provide new insight into bacterial diversity under unfavourable conditions, new isolates and probably new genetic information on heavy metal resistance, which could be exploited in revegetation in future (Fabienne et al., 2003).

In this paper, a tailing sample was collected from the 4 # tailing of Dexing copper mine, Jiangxi province, China. Dexing copper mine is a super-scaled copper mine in China (Chen et al., 2005) and also the biggest opencast copper mine in Asia. It has a history over 1000 years and has been mined since 1965. The 4 # tailing is the biggest tailing in China and reclamation is undeveloped. The aim of this study was to isolate indigenous bacterial strains with high heavy metal resistance in the tailing site. The

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physiological and biochemical features were used to characterize the strains. Phylogenetic analysis, based on 16S rDNA gene sequence data, was also used to reveal the genetic relationship between the strains and others.

MATERIALS AND METHODS

Site description

Dexing copper mine is located in the Sizhou town (latitude/ longitude: 29°43′ N/ 117°02′ E) of the Dexing city, Jiangxi province, South China, at an altitude range of 65 to 500 m, covering an area of about 100 km^2. This area has a subtropical monsoon climate. Average annual temperature and precipitation are about 17°C and 1 900 mm, respectively. The 4 # tailing pool is still under working, with a designed storage of 8.35×10^9 m^3, covering an area of 14.3 km^3. Approximately 100, 000 metrics tons of flotation tailing were deposited into the pool pile per day. The 4 # tailing pool is the biggest one in Asia and reclamation is undeveloped.

Direct isolation of heavy metal resistant strain by plating

The tailing sample was obtained from 4 # tailing dam slope, in May 2007. To obtain soil bacterial supernatant, 2 g soil was weighed into 50 ml flask, mixed with 20 ml distilled water and then put in a rotating shaker with a speed of 150 rpm at 30°C for 30 min. 0.1 × yeast tryptophan peptone glucose (YTPG) agar plates (each per liter: 0.25 g peptone, 0.25 g tryptone, 0.5 g yeast extract, 0.5 g glucose, 30 mg $MgSO_4 \cdot 7H_2O$, 3.5 mg $CaCl_2 \cdot 2H_2O$, 15 g agar) with 300 µg/ml cycloheximide (Sigma-Aldrich Co., USA), supplemented with heavy metal at the following concentrations: Cd $(NO_3)_2 \cdot 4H_2O$, 0.1, 0.5, 1.0, 1.5, 2.0 mM; $ZnSO_4 \cdot 7H_2O$, 0.1, 0.5, 1.0, 1.5, 2.0 mM, were used to isolate cadmium and zinc tolerant bacteria strains, respectively. 0.1 ml of the liquid soil bacterial supernatant was plated on each disk. Plates were incubated at 30°C for 3 – 7 days (Yuko and Naoyuki, 2007). The formed colonies were transferred to plates supplemented with higher heavy metal concentrations: cadmium ranges from 2.0 to 12 mM and zinc ranges from 2.0 to 55 mM to screen highly cadmium tolerant and zinc tolerant strains, respectively. The strain DX-T3-01 performed well in screening tests and had the greatest resistance to heavy metal cadmium. The strain DX-T3-03 had the greatest resistance to zinc. They were enriched and transferred to 0.1 × YTPG liquid mediums with heavy metal concentrations: cadmium ranged from 2.0 to 30 mM and zinc from 2.0 to 60 mM, with an inoculation of 5% (v: v), to obtain highly cadmium resistant culture and zinc resistant culture, respectively. The flasks were incubated at 30°C and agitated at 150 rpm.

Morphology

The enriched bacterial cells were collected. Olympus CX 31 optical microscope and JEOL-5600SL scanning electron microscope (SEM) were employed for counting and morphological examination.

Effects of different heavy metals on the growth of bacteria strains

Stationary-phase cells of DX-T3-01 and DX-T3-03 were inoculated into 0.1 × YTPG liquid medium supplemented with different heavy metal ions, respectively. The concentrations of heavy metal in the 0.1 × YTPG medium were: $ZnSO_4 \cdot 7H_2O$, 0.0, 1.0, 2.0, 4.0, 6.0, 8.0, 10, 15, 20, 25, 30, 35, 40 mM; $CuSO_4 \cdot 5H_2O$, 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 mM; $Cd(NO_3)_2 \cdot 4H_2O$, 0.0, 2.0, 4.0, 6.0, 8.0, 10, 12, 14, 16, 18 mM; $C_4H_6O_4Pb \cdot 3H_2O$, 0.0, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 mM; $NiSO_4 \cdot 6H_2O$, 0.0, 0.1, 0.5, 1.0, 2.0, 3.0 mM. The cultures were incubated for 24 h at 30°C and agitated at 150 rpm. Growth rates of the strains were determined by absorbance at wavelength of 600 nm (OD_{600}) with a HITACHI U-2910 spectrophotometer. Each sample was done in triplicate.

Physiological and biochemical characteristics

Physiological and biochemical characteristics, including temperature and pH range for growth, tolerance of different NaCl concentrations and resistance to antibiotics were tested. Utilization of different carbon sources such as sugars, organic acids and amino acids were analyzed using BIOLOG identification system (BIOLOG Microstation™, Biolog Inc., Hayward, CA). The GEN □ microplates were prepared and inoculated according to the BIOLOG manufacturer's directions. Microplates were covered and incubated at 33°C. The plates were read with a BIOLOG microplate reader (590 nm) at 22 - 24 h.

16S rDNA

DNA extraction and purification of the bacterium were done according to the protocols described by Zhou et al. (1996). 16S rDNA genes were amplified by polymerase chain reaction (PCR) in 50 µl mixtures according to previously studies (Liu et al., 2007). In the reaction, the primers sequences of bacteria-specific 27F and the universal 1492R were 5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-CGGCTACCTTGTTACGACTT-3′, respectively (Lane, 1991; Liu et al., 2007). A gene amplifier (Biometra, T-Gradient, Genman) was used to incubate reactions through an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 40 s, 55°C for 30 s and 72°C for 1 min and finally at 72°C for 10 min. The PCR products with expected size of about 1.5 kb were pooled and purified using Promega PCR purification columns according to the manufacturer's instructions.

The 16S rDNA were sequenced by 3730 automatic sequencing equipment (Invitrogen Corporation). Phylogenetic affiliation of the 16S rDNA sequence was initially estimated using the program BLAST-a basic search tool of the National Center for Biotechnology Information (http://www.ncbi. nln.nih. gov/BLAST). Based on the phylogenetic results, appropriate subsets of 16S rDNA sequences were selected and subjected to a final phylogenetic analysis with CLUSTAL V, phylogenetic development trees were built (Liu et al., 2007).

Nucleotide sequence accession numbers

The 16S rDNA sequences have been submitted to GenBank with accession numbers: strain DX-T3-01 (GQ895735), strain DX-T3-03 (GQ895737).

RESULTS

Isolation by plating

After incubation at 30°C for 3 -10 days, colonies formed on each plate. The images of colonies of bacterial strains, DX-T3-01 and DX-T3-03, on plates are shown in Figures 1 and 2, respectively. The colonies of strain DX-T3-01 are round and with white color and wet smooth surface (Figure 1). They can grow well on solid medium supple-

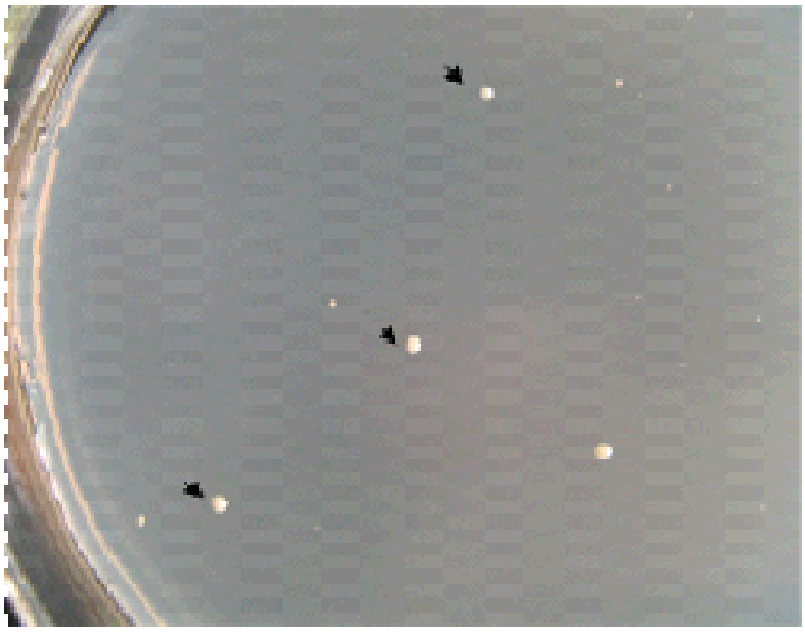

Figure 1. Colonies on solid medium, supplemented with cadmium at the concentration 10 mM. Arrows indicate the white colonies of strain DX-T3-01.

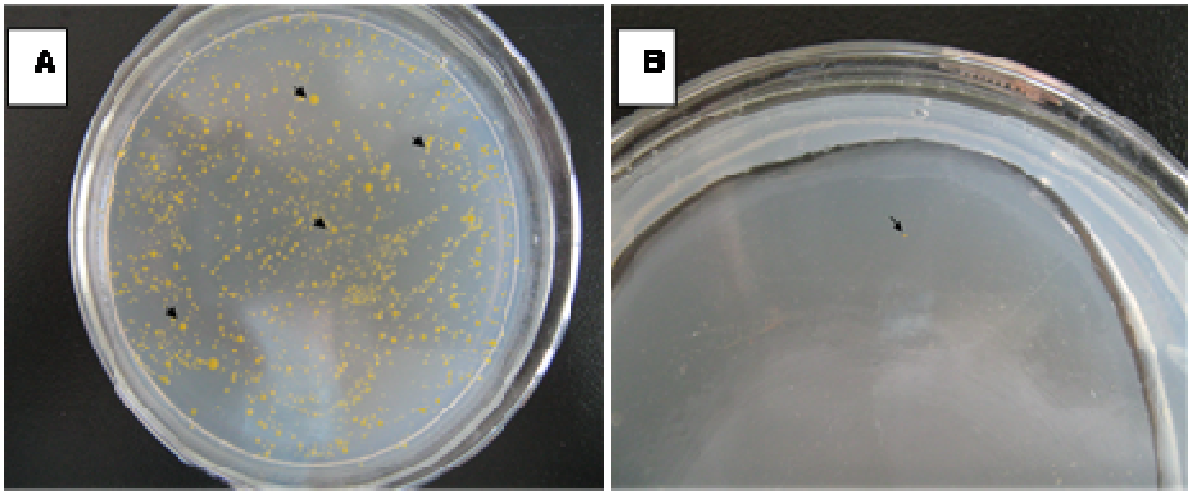

Figure 2. Colonies on solid medium, supplemented with zinc at high concentrations. **A** - 35 mM; **B** - 50 mM. Arrows indicate the yellow colonies of strain DX-T3-03.

mented with cadmium concentration as high as 10 mM after being incubated for 7 days. Figure 2 shows the growth of strain DX-T3-03 on solid mediums supplemented with zinc. The colonies of strain DX-T3-03 are round, with yellow color and wet smooth surface. They can grow well on solid medium supplemented with zinc as 35 mM after being incubated for 3 days (Figure 2 A) and can grow on solid medium with zinc concentration as high as 50 mM after incubation for 10 days (Figure 2 B).

After several generations' cultivation with heavy metals, the strain DX-T3-01 showed high tolerance to cadmium. It could grow well in 0.1 × YTPG liquid medium with cadmium concentration of 18 mM (OD_{600nm} is 0.242, the counting of cell number is 4×10^7cell/ml) and it can live in liquid medium with cadmium concentration up to 30 mM (OD_{600nm} 0.025, counting of cell number 4×10^6cell/ml). The strain DX-T3-03, which had high tolerance to zinc, can grow well in liquid medium with zinc of 40 mM (OD_{600nm} is 0.291, counting of cell number 7×10^7cell/ml). It can live in liquid medium with zinc concentration up to 50 mM (OD_{600nm} 0.055, counting of cell number 8×10^6cell/ml).

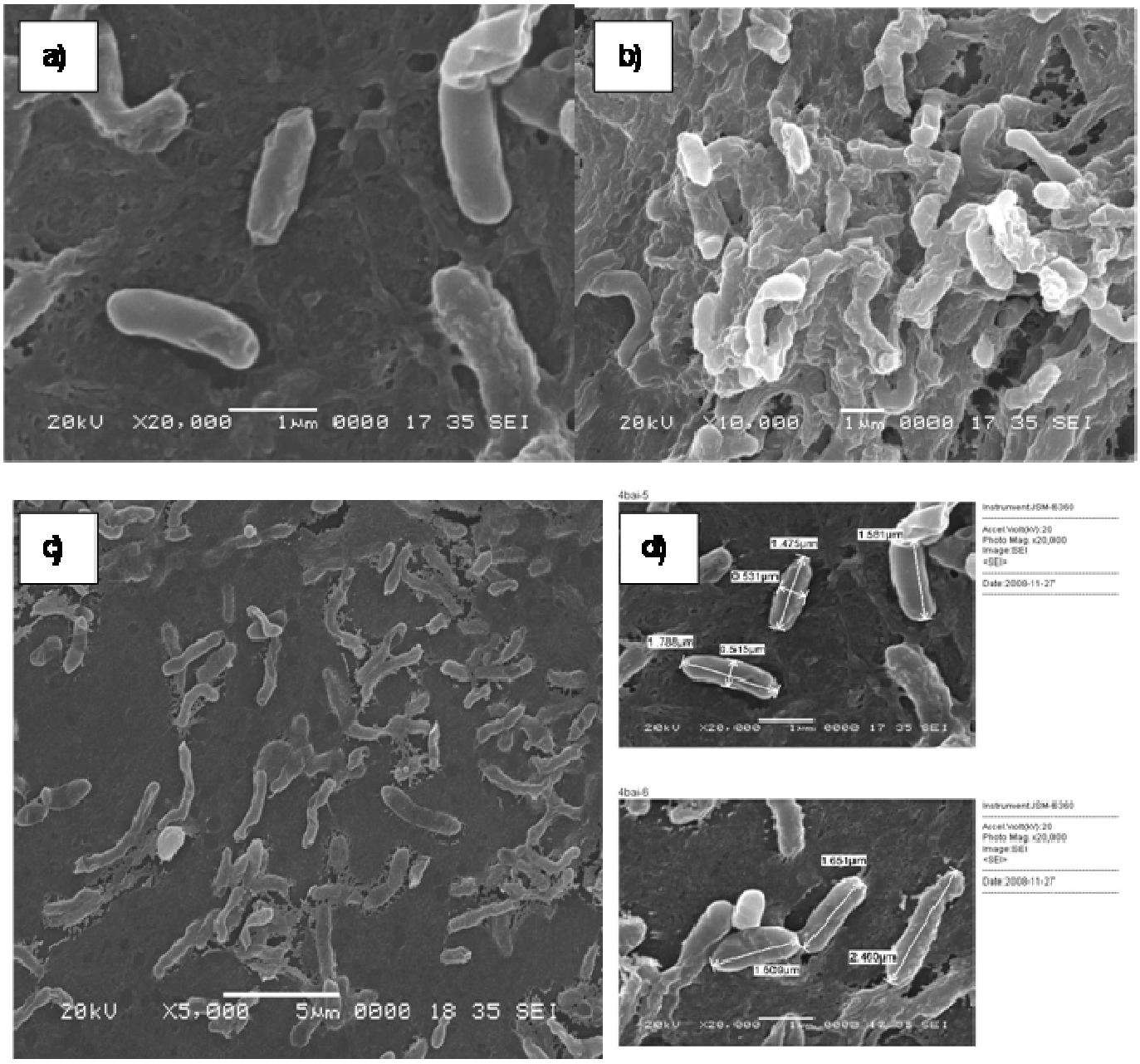

Figure 3. SEM images of bacterial strain DX-T3-01 at different magnifications**. a**) ×20,000; **b**) ×10,000; **c**) ×5,000; **d**) ×20,000, size measurement.

Morphology of bacteria

The bacterial morphology observed by scanning electron microscope are shown in Figures 3 and 4, respectively. It can be seen that strains DX-T3-01 and DX-T3-03 were *Bacillus*. The cell size of strains DX-T3-01 and DX-T3-03 were measured. Cells of strain DX-T3-01 were 0.5 - 2.0 × 1.0 - 8.0 μm in size, while cells of strain DX-T3-03 were in smaller size of 0.5 - 2.0 × 1.0 - 4.0 μm. Cells of strain DX-T3-01 can secrete much sticky secretion covering themselves.

Growth of strains DX-T3-01 and DX-T3-03 in the presence of heavy metals

Figures 5 and 6 showed the growth of strains DX-T3-01 and DX-T3-03 in 0.1 × YTPG liquid medium in the presence of other heavy metals. It can be seen that strain DX-T3-01 had tolerance to zinc (10 mM), copper (3.0 mM), lead (0.75 mM) and nickel (1.0 mM) (Figure 5). While strain DX-T3-03 had tolerance to heavy metals: cadmium 2 mM, copper 1.5 mM, lead 1.0 mM and nickel 1.0 mM (Figure 6).

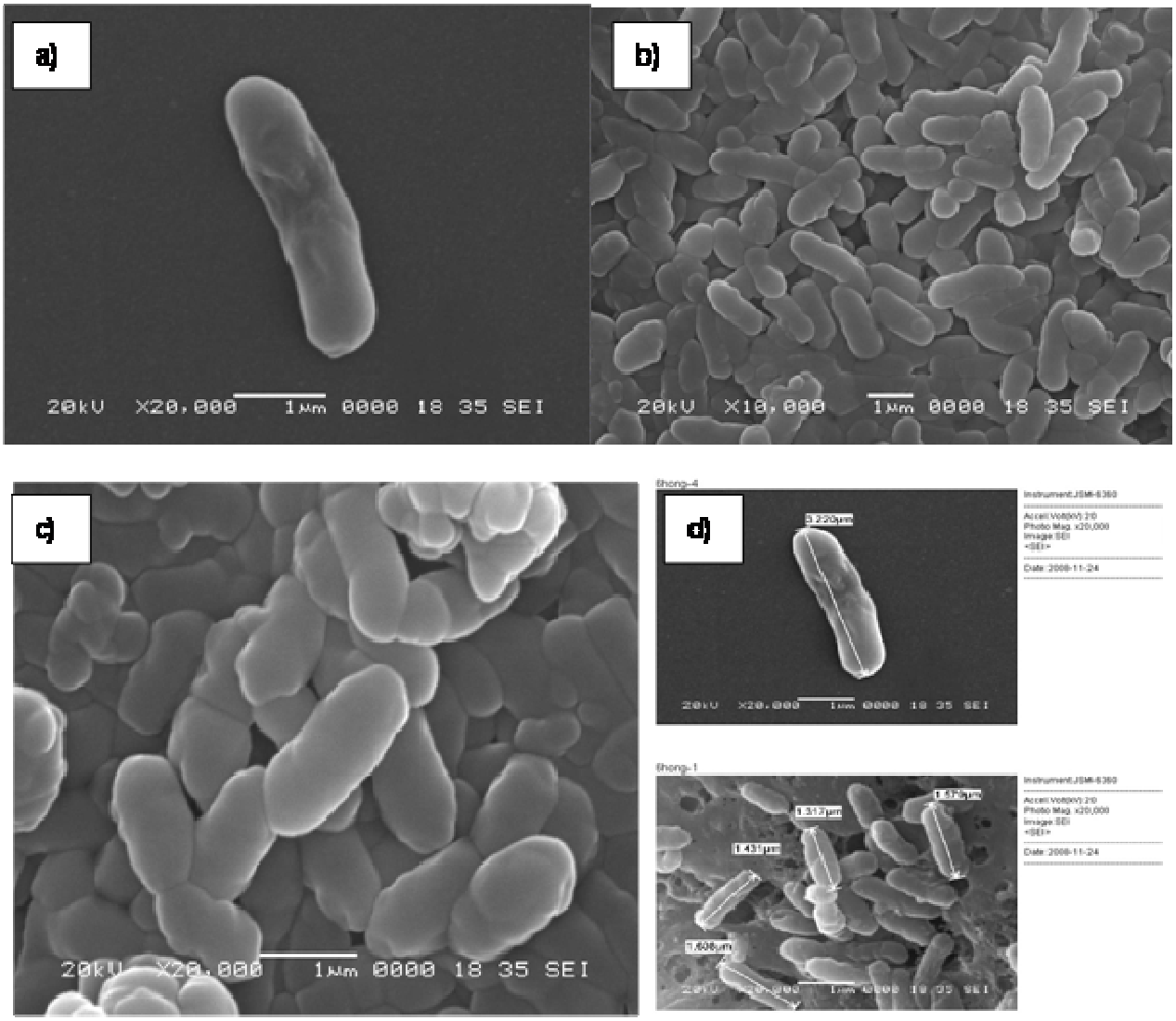

Figure 4. SEM images of bacterial strain DX-T3-03 at different magnifications.a) ×20,000; b) ×10,000; c) ×20,000; d) ×20,000, size measurement.

Physiological-biochemical characteristics

The physiological-biochemical characteristics of strains DX-T3-01 and DX-T3-03 are shown in Table 1. The strain DX-T3-01 had a wide growth temperature from 20 - 45℃ and the optimal growth temperature is 35 - 37℃; the pH range is from 4.0 to 10.0 and the optimal pH is 6.5 - 7.0; salt tolerance was up to 5% NaCl. DX-T3-01 was resistant to the following antibiotics: Chloramphenicol (5 - 50 μg/ml), streptomycin (5 - 250 μg/ml) and ampicillin (50 - 1500 μg/ml). DX-T3-01 could use the following main carbon sources: Sucrose, D-maltose, D-trehalose, D-cellobiose, dextrin, α-D-glucose, D-mannose, D-fructose, D-galactose, L-fucose, L-rhamnose, D-sorbitol, D-mannitol, rifamycin SV, minocycline, pectin, nalidixic acid, potassium tellurite, aztreonam, sodium butyrate, sodium bromate and D-serine.

The strain DX-T3-03 had similar growth temperature, pH and salt tolerance, such as growth temperature from 20 to 37℃ and the optimal growth temperature of 35 - 37℃; pH range is from 2.0 to 12.0, optimal pH at 6.5 - 7.0; salt tolerance was up to 5% NaCl. But DX-T3-03 was more resistant than the strain DX-T3-01 to the following antibiotics: Chloramphenicol (5 - 60μg/ml), streptomycin (50 - 1000μg/ml) and ampicillin (50 - 2000 μg/ml). DX-T3-03 could use the following main carbon sources: α-D-glucose, D-fructose, L-lactic acid, citric acid, D-galactose, rifamycin SV, potassium tellurite, aztreonam, l-alanine, l-aspartic acid, l-pyroglutamic acid.

Phylogenetic analysis of 16S rDNA

The 16S rDNA of bacterial strains DX-T3-01 and DX-T3-

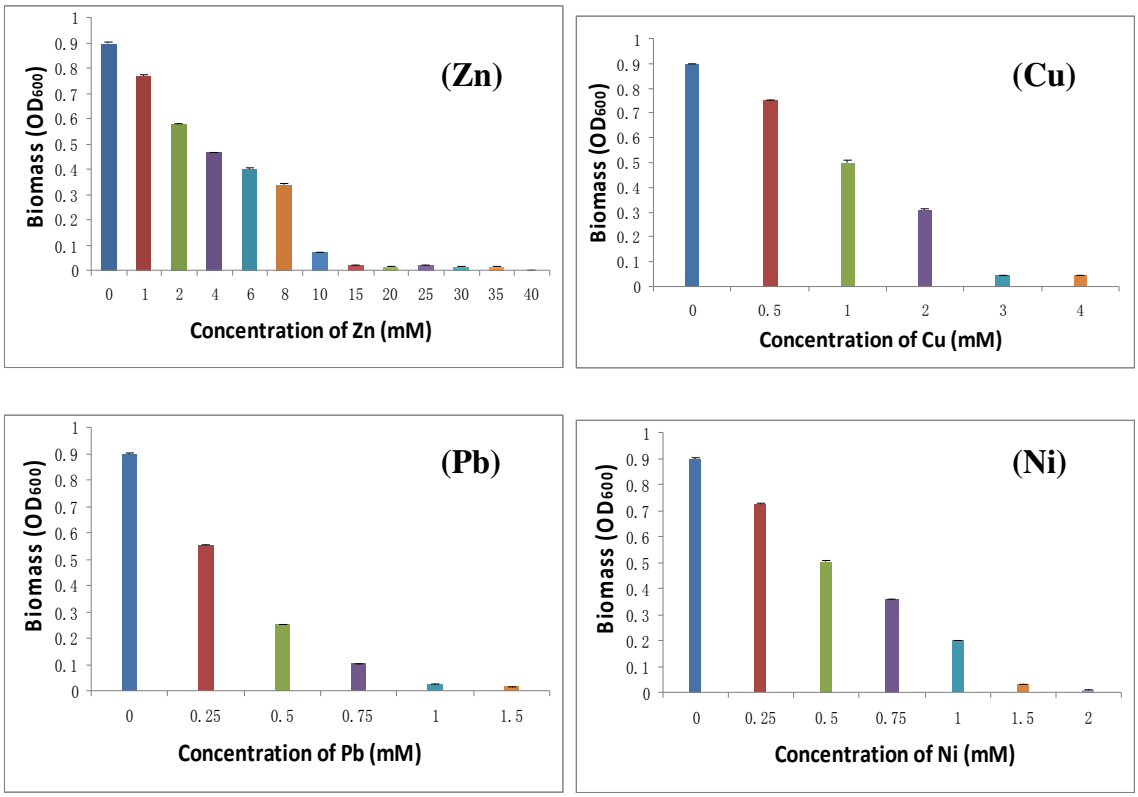

Figure 5. Growth of strain DX-T3-01 in the presence of different heavy metals. Error bar stands for standard deviation (SD).

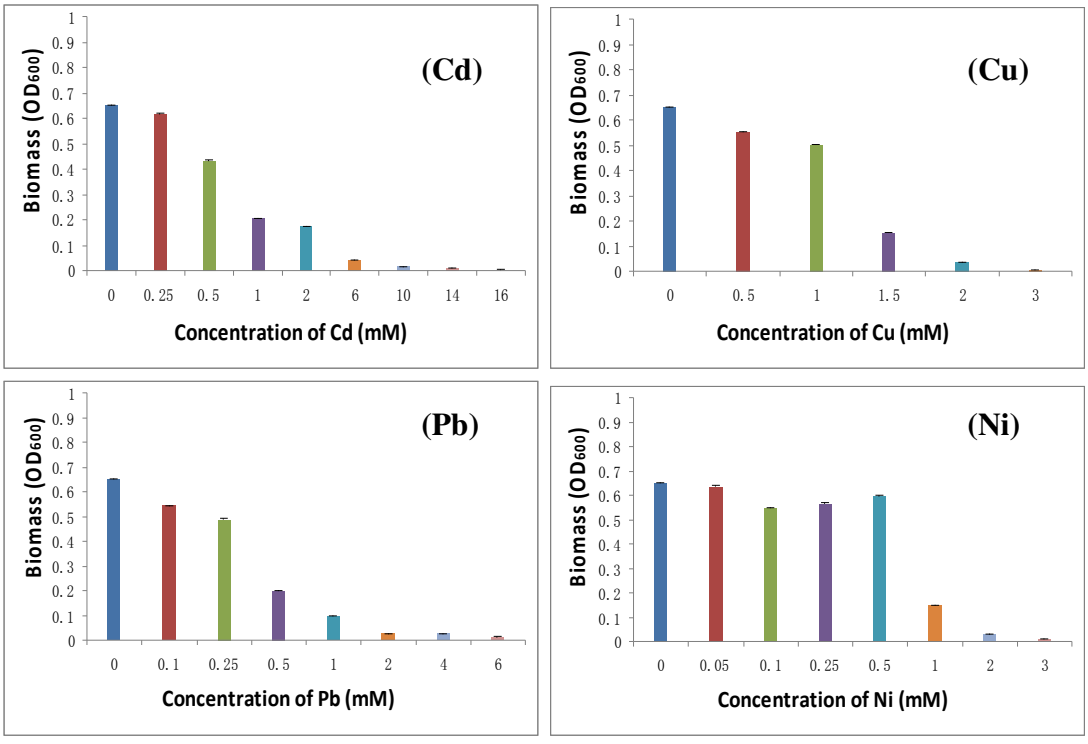

Figure 6. Growth of strain DX-T3-03 in the presence of different heavy metals. Error bar stands for standard deviation (SD).

Table 1. The physiological and biochemical characteristics of strains DX-T3-01 and DX-T3-03.

Parameters	Strain	
	DX-T3-01	DX-T3-03
Physiological characteristics		
Temperature (℃)		
20	+	+
25	+	+
30	+	+
35	++	++
37	++	++
40	+	-
45	+	-
50	-	-
Growth at pH		
2	-	+
4	+	+
6	+	+
6.5	++	++
7	++	++
8	+	+
10	+	+
12	-	+
Growth on NaCl (%)		
1	+	+
2	+	+
5	+	+
6	-	-
7	-	-
Antibiotics resistance		
Chloramphenicol	5, 25, 50 μg/ml	5, 25, 50, 60 μg/ml
Streptomycin	5, 50, 100, 200, 250 μg/ml	50, 100, 300, 500, 800, 1000 μg/ml
Ampicillin	50, 200, 400, 800, 1200, 1500 μg/ml	50, 200, 500, 1000, 1500, 2000 μg/ml
Main carbon source in BIOLOG GEN III microplates		
Sucrose	+	-
D-Maltose	+	-
D-Trehalose	+	-
D-Cellobiose	+	-
Dextrin	+	-
α-D-Glucose	+	+
D-Mannose	+	-
D-Fructose	+	+
L-Lactic Acid	-	+
D-Galactose	+	+
L-Fucose	+	-
L-Rhamnose	+	-
D-Sorbitol	+	-
D-Mannitol	+	-
Rifamycin SV	+	+
Minocycline	+	-
Pectin	+	-
Nalidixic acid	+	-
Potassium tellurite	+	+

Table 1. Contd.

Citric acid	-	+
Aztreonam	+	+
Sodium butyrate	+	-
Sodium bromate	+	-
L-Alanine	-	+
L-Arginine	-	-
L-Serine	-	-
L-Aspartic acid	-	+
L-Pyroglutamic acid	-	+
D-Serine	+	-

++, Grow well; +, growth; -, no growth.

03 were amplified with primers 1492R and 27F. The PCR amplification products were detected by 0.8% agarose gel electrophoresis with ultraviolet (UV). The length of object fragment is about 1, 500 bp.

Sequence analysis of the 16S rRNA gene has been considered a fast and accurate method to identify the phylogenic position of bacteria. Full-length 16S rDNA of strains DX-T3-01 and DX-T3-03 were sequenced and used to construct phylogenetic development trees (Figure 7 A and B). We found that strain DX-T3-01 was classified in the branch of *Ralstonia* sp. and it had 99% similarity to *Ralstonia pickettii* RAL01 LMG 7160, *R. pickettii* strain 2000030791 (similarity 99%), *Ralstonia* sp. JB1B3 (similarity 99%), *Ralstonia thomasii* strain LMG6866 (similarity 98%), *Ralstonia mannitolilytica* strain AU428 (similarity 98%) and uncultured *Ralstonia* sp. clone GBR-C-30 (similarity 95%) (Figure 7 A). The strain DX-T3-03 belongs to the *Sphingomonas* sp. branch and it had 99% similarity to *Sphingomonas* sp. JSS-7, *Sphingomonas melonis*, *Sphingomonas* sp. CSSB-2, *Sphingomonadaceae* bacterium KVD-unk-19, uncultured soil bacterium clone F43_Pitesti, uncultured soil bacterium clone F20_Pitesti and *Sphingomonas* sp. EBD (Figure 7 B). The two strains were named as *R. pickettii* strain DX-T3-01 and *Sphingomonas* sp. strain DX-T3-03, respectively.

DISCUSSION

The range of parameters which *Escherichia coli* is able to tolerate (Cd 0.5 mM; Zn 1.0 mM; Cu 1.0 mM; Pb 5.0 mM and Ni 1.0 mM [Nies, 1999]) is not considered extreme, whereas parameters well beyond this range may be considered as extreme (Nies, 2000). By using the *E. coli*-based definition, extreme environments contaminated with heavy metals are easily defined and bacteria able to grow in such environment may thus be designated "metallophile" bacteria (Nies, 2000). In our study, strains DX-T3-01 and DX-T3-0 were isolated from the long-term heavy metal-polluted mine tailing and they could be tolerant to heavy metal concentrations well beyond the minimal inhibitory concentration of *E. coli*, and thus, could be considered as "metallophiles".

Many indigenous organisms isolated from heavy metal contaminated sites had tolerance to heavy metals toxicity. The metal resistance of *Ralstonia* in lake sediments and industrial biotopes has previously been reported (Diels and Mergeay, 1990; Yong et al., 2008). *Ralstonia* sp. CH34, formerly *Alcaligenes eutrophus* (Brim et al., 1999), is the best known example of heavy metal-resistant bacterial strains, which had heavy metal ions minimal inhibitory concentrations (mM) as follows: Co^{2+} 20 mM, Zn^{2+} 20 mM, Cd^{2+} 5 mM. Duan and Min (2004) isolated a strain DKC1 of *Ralstonia eutropha* from a long-term cadmium treated soil sample. They found it had highly resistance to cadmium, could grow well on solid medium supplemented with 3 mM cadmium. Strain DKC1 had high resistance to Cd but lower resistance to other heavy metals. The strain had a big plasmid, whose Cd-resistance genes are located on its plasmid. *Ralstonia metallidurans* CH34, a phenol-degrading strain with anti-heavy metal feature by immobilization. The phenol-degrading efficiency of immobilized *Ralstonia metallidurans* CH34 was investigated by Wu et al. (2005). Results showed that phenol-degrading efficiency of immobilized cells is obviously superior to that of the free cells and the ability of its resistance to heavy metal has been greatly improved. *R. pickettii*, a newly designed genus by Yabuuchi et al. (1995), is an aerobic gram-negative, nonfermentative rod and is a ubiquitous microorganism found in water and soil. This species has demonstrated resistance to heavy metals, such as cadmium, copper and zinc and showed the ability to breakdown many toxic organic substances. Mark et al. (2000) obtained a strain of *R. pickettii* that could grow in medium with 0.819 mM Cd^{2+}. Gilotra and Srivastava (1997) isolated a train of *R. pickettii* from soil, it could grow on medium with heavy metals Cd^{2+}, Cu^{2+} and Zn^{2+} concentration as high as 2, 2 and 25 mM, respectively. In the present study, the *R. pickettii* strain DX-T3-01 that had outstanding tolerance to heavy metal cadmium (10 mM on solid medium and 18 mM in liquid medium), should be considered as "high cadmium tolerant

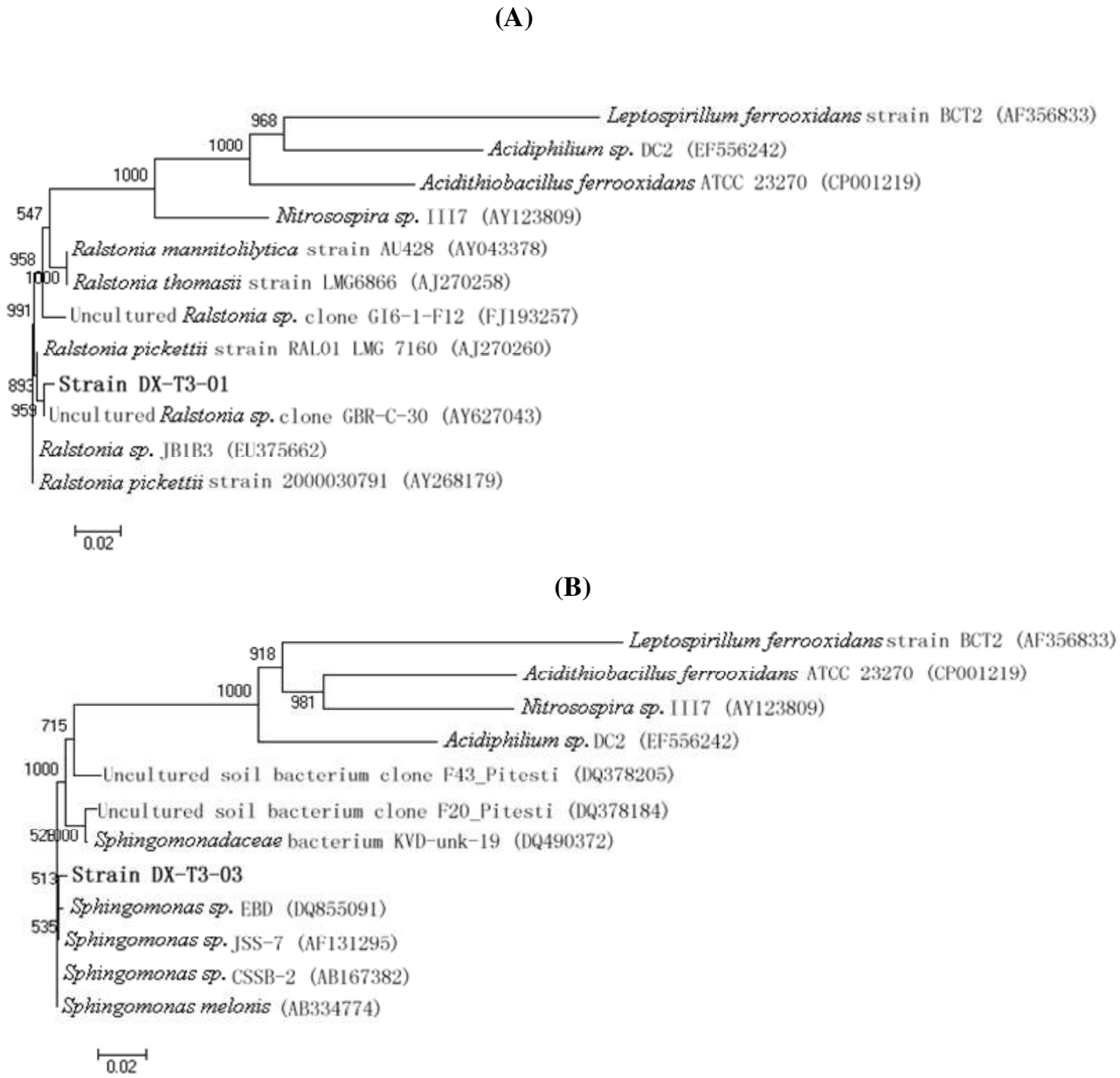

Figure 7. Phylogenetic development trees based on 16S rDNA ananlysis. (**A**)- bacterial strain DX-T3-01; (**B**)- bacterial strain DX-T3-03.

bateria".

Sphingomonas is a novel bio-resource and widely spread in various aquatic and terrestrial environments. Yabuuchi et al. (1990) described the characteristics of this genus for the first time and Takeuchi et al. (1993) amended it. *Sphingomonas* belonged to α-*proteobacteria,* gram-negative, but is some different from the typical gram-negative species for not having lipopolysaccharide. Glycosphingosides exists in its cell membrane, while the glycosphingosides is usually detected as a component in eukaryote cell membrane. Investigations indicated that *Sphingomonas* species could metabolize many kinds of toxic polycyclic aromatic hydrocarbons like dimethyl-benzene, biphenyl, toluene, naphthaline, pesticide carbofuran and naphthalenesulfonate (Baraniecki et al., 2002). More recently, several *Sphingomonas* strains with the ability to degrade organic compounds such as dyes and microcystic toxins have been described (Cao et al., 2003; Jones et al., 1994). Therefore, it has great potential for applications in environmental bioremediation. Some strains of *Sphingomonas* species have been isolated from heavy metal polluted soils around industry factories. Sun et al. (2009) isolated copper tolerant strains *Sphin-gomonas* sp. (JM14, YM22 and YM12) from copper

contaminated soils. Tangaromsuk et al. (2002) investigated and showed that strain of *Sphingomonas paucimobilis* had high tolerance to heavy metal Cd, the minimal inhibitory concentration was 1.5 mM. The strain had strong adsorption ability to Cd and the efficiency of removal of Cd from water was up to 84%. Hyo et al. (2007) studied the influence of heavy metals to the degradation of dibenzofuran by *Sphingomonas wittichii* RW1. The results showed that heavy metals Cd, Hg and Cu could significantly restrain the degradation process, while As and Pb almost had no effect on the strain. Different from the present reports, the *Sphingomonas* sp. strain DX-T3-03 had high resistant ability to many heavy metals such as cadmium (2 mM), copper (1.5 mM), lead (1.0 mM) and nickel (1.0 mM) and the most remarkable thing is its significant tolerance to heavy metal zinc (35 mM on solid medium and 40 mM in liquid medium), which should be considered as "super zinc tolerant bateria".

Both Cd and Zn are considered as one of the most toxic heavy metals and they can appear either in water or soil of any polluted site because of their high mobility, especially in agricultural fields, thus greatly threatens human health from food chains (Goris et al., 2001). Biosorption of these metals with the use of indigenous microorganism is efficient and ecofriendly for its immobilization. The immobilizations of Zn and Cd by indigenous and augmented microorganisms had been reported by Yong et al. (2008). The application of metal-resistant bacteria for bioremediation offers attractive perspectives (Mergeay et al., 2003). The two indigenous strains (*R. pickettii* strain DX-T3-01 and *Sphingomonas* sp. strain DX-T3-03) obtained from the mine tailing in this study, owing to their excellent resistance for toxicity by heavy metals, will be good candidates for tailing bioremediation studies in the future.

In summary, we have isolated two highly heavy metal resistant bacterial strains (DX-T3-01 and DX-T3-03) from the tailing sample. They showed significant heavy metal tolerance to cadmium and zinc, respectively. Their physiological-biochemical characteristics were investigated and the utilization of carbon sources analyzed showed their diversity of metabolic patterns. Based on 16S rDNA sequence analysis, they were identified as *R. pickettii* strain DX-T3-01 and *Sphingomonas* sp. strain DX-T3-03, respectively. This study supplied potential bacterial materials for bioremediation of heavy metal pollution for tailing in the future. Besides, the degradation of toxic organic substances such as pesticide by these two strains is being studied, which offer attractive perspectives in future bioremediation of the combined contamination including both heavy metal and organic pollutants in agriculture soils around the tailing site.

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