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Full Length Research Paper

Bacterial community composition in reclaimed and unreclaimed tailings of Dexing copper mine, China

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An investigation was conducted to compare the microbial community structures between contaminated and reclaimed mine tailings of Dexing copper mine, Jiangxi province, China. Sample T1 was obtained from tailings site, where reclamation of phytostabilization has been conducted for 20 years successfully whereas, the other one (sample T4) was obtained from the unreclaimed tailings site. Physico-chemical characteristics, chemical speciation of heavy metals Cu, Cd and Zn were revealed to compare the effects of reclamation. A polymerase chain reaction (PCR)-based cloning approach was employed to investigate the bacterial community composition in these two samples. Results indicate the improved physico-chemical conditions and decreased bioavailability of heavy metals Cd and Zn significantly in the sample collected from reclaimed site compared with the other unreclaimed mine tailings site. Phylogenetic analysis indicated that bacteria in these two samples fell into 12 putative phylogenetic divisions. Sample T1 had 12 phyla were present, and the community was predominated by *Planctomycetes* (20%), α-*proteobacteria* (16%), *Chloroflexi* (12%) and *Acidobacteria* (10%). On the other hand, sample T4 had only 4 phyla and was predominated by γ-*proteobacteria* (86%). The diversity of microbes in sample T1 significantly increased when compared with sample T4. The structure of microbial community composition in the first sample also was optimized to sustain more phyla associated with a healthier soil after reclamation. From the results, it could be deduced that the inventories of bacterial populations such as *Planctomycetes, Chloroflexi,* α-*proteobacteria*, *Acidobacteria* and *Actinobacteria* predominated in sample T1, but were essentially absent in sample T4. This may serve as potential bioindicator for the reclamation evaluation of tailings.

Key words: Mine tailings, reclamation, bacterial community composition, bioindicators.

INTRODUCTION

The mining activity has produced a large amount of metal-rich wastes, caused serious environmental pollution and soil degradation in the world (Sheoran and Sheoran, 2006; Li et al., 2009). In China, the metal ore mining and processing generated 242.2 × 10^6 t tailings in 2002, and over 6336 × 10^6 t solid waste storage has been accumulated (Li, 2006). The degraded land associated with mining account for 3.2 × 10^6 km^2, 80 to 90% of which still remain unreclaimed (Li et al., 2009).

Recent interest in the reclamation of abandoned mine tailings focuses on re-vegetation or phytostabilization (Munshower, 1994; Monica et al., 2008). The ultimate goal of phytostabilization is to attain the plant species richness associated with ecosystems above and below the soil stability and resilience (Mummey et al., 2002; Monica et al., 2008). Many studies have emphasized a strong association between the establishment of a stable plant community and the abundance and composition of soil microorganisms (Moynahan et al., 2002; Londry and Sherriff, 2005; De La Iglesia et al., 2006; Monica et al., 2008). These organisms may be important for re-vegetation after a significant reduction in the soil metal concentration has been achieved (Pawloska et al., 2000; Fabienne et al., 2003). Besides, it may provide new insight into bacterial diversity under unfavorable heavy metal-polluted conditions; potential biomarker, new iso-

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isolates and probably new genetic information on heavy metal resistance could be exploited (Fabienne et al., 2003). So, it is very important to study the soil organisms indigenous in different tailings, especially in reclaimed tailings sites.

Dexing copper mine, is a super-scaled copper mine in China. It is also the biggest opencast copper mine in Asia (Chen et al., 2005). For more than 40 years mining, accumulated large amount of tailing became one of its main environmental problems. Since 1990s, the reclamation through phytostabilization has been conducted on its one tailings site, and till now, it has been successfully re-vegetated for nearly 20 years. The other tailings site, which has the biggest tailings pool and dam in Asia, is still under work and will need reclamation. In order to conduct a comprehensive phylogenetic comparison of microbial community composition present in reclaimed and unreclaimed tailings sites, a PCR-based cloning approach (restriction fragment length polymorphism, RFLP) was adopted. Then, the relative compositions of the bacterial communities were compared in the light of physicochemical characteristics and metals bioavailability of the tailings sites. The results obtained in this study may enrich knowledge of microbial community in different mine tailings environments, and may provide information concerning potential bioindicators for reclamation evaluation of tailings in future.

MATERIALS AND METHODS

Sites 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 from 65 to 500 m, covering an area 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. Rainfall is highest in summer (April to June); average monthly rainfall is 200 to 300 mm. The 1# tailings pool has in storage of about 2.15×10^7 m^3 of flotation tailings, covering an area of 1.21 km^2 after running out of service in 1986. Beginning from the late 1990s, a series of experiments were performed on re-vegetation of a part of the mine tailings pool dam slope, 30 hm^2 in acreage, which was covered with guest soil. Different vegetations such as peanut (*Arachis* Linn.), vetiver (*Vetiveria zizanioides* Nash) and slash pine (*Pinuselliottii Engelm*) were planted on the dam slope (Chen et al., 2005). The 4# tailings pool has a designed storage of 8.35×10^9 m^3, covering an area of 14.3 km^2. Approximately 10^4 metric tons of flotation tailing were deposited into the pile per day. It has the potential to pollute water and agricultural soils downstream of the dam. No reclamation has been undertaken at this site.

Soil sampling and preparation

Samples were taken in May 2008. Sample T1 was obtained from the dam slope of the 1# tailings site, which grows re-vegetations. Sample T4 was collected from the top surface of the 4# tailings dam. Each sample included six cores of 5 cm in diameter and 15 cm deep (each soil core about 500 g); were taken randomly from different areas at each tailings site. Each replicate soil sample was mixed thoroughly (Wang et al., 2007). Field moist soils were sieved (< 2 mm) by nylon sieve and large pieces of plant material and stones were removed. Part of the samples was kept moist in the dark at 4°C for preparation. The remaining soil was stored at -80°C to extract soil DNA. Sub-samples were air-dried at ambient temperature for 72 h, disaggregated and sieved to an adequate size for further analysis (< 0.2 mm or < 74 µm). Due to the strong association and affinity of heavy metals with fine grained soil components, the < 74 µm soil fraction was used for the sequential extraction and total acid digestion methods (Rodrıíguez et al., 2009). The < 0.2 mm soil fraction was used to analyze pH, organic carbon, organic matter content and cation exchange capacity (CEC). The water content of the dried samples was calculated by heating a sub-sample in an oven at 105 ± 2°C to constant weight (Rodrıíguez et al., 2009). All experiments were performed in triplicate and the average values were reported on a dry weight basis.

Soil analysis

The total concentrations of metals Zn, Fe, Cu, Pb, Cd, Cr, Hg, As, Mn, Ag, Au, Co, Ni, Mg, Ca, Al and elements of K, S, P were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Prodigy, Leeman ABS INC. USA). A 1:2.5 (m:v) soil-water suspension was prepared and left standing overnight for pH measurement with a pH meter PHSJ-4A (Abollino et al., 2002). Organic carbon was determined by Walkley-Black method (Abollino et al., 2002). Considering that the average content of carbon in soil organic matter is equal to 58%, the conversion factor 1.724 was used to calculate the percentage of organic matter from the content of organic carbon (Abollino et al., 2002). The CEC was determined with barium chloride (Bartels, 1996; Abollino et al., 2002). Particulate-bound Cu, Cd and Zn fractions in the soils were determined following the Tessier's sequential extraction scheme (Tessier et al., 1979, 1980). Concentrations of the extracts obtained were analyzed by flame atomic absorption spectrophotometer Z-2000 (Hitachi, Japan). All experiments were performed in triplicate and the average values were reported.

DNA extraction and purification

Extraction of nucleic acids from the two soil samples was according to the procedure described by Zhou et al. (1996). The crude DNA was purified by using Wizard plus sv Minipreps DNA purification system (Promega Corporation, USA) and quantified by ethdium bromide-UV detection on an agarose gel.

PCR and fractionation of 16S rDNA genes

Bacterial 16S rDNA genes were amplified with the primer set as 1492R (50- CGGCTACCTTGTTACGACTT-30) and 27F (50-AGAGTTTGATCCTGGCTCAG-30) (Lane, 1991). A PCR amplifier (Biometra, T-Grandient, Germany) 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 was completed with an extension period of 10 min at 72°C. Products from the amplification reactions of expected size (about 1500 bp) were pooled and purified before ligation.

Cloning, restriction fragment length polymorphism (RFLP) and sequencing

The purified PCR products were ligated to the vector PGEM-T (Promega Corporation, USA), and then transformed to *E. coli* DH5α competent host cells. More than 120 white colonies were randomly

Table 1. Elements' concentrations in the two samples.

Sample	Element (mg/kg)									
	Zn	Fe	Cu	Pb	Cd	Cr	Hg	As	Mn	Ag
T1	22.8	25000	106.2	17.4	1.7	40.1	0.04	8.8	186.3	0.7
T4	44.6	31600	452.4	37.7	2.7	45.8	0.05	11.8	373.2	0.7
	Au	**Co**	**Ni**	**Ca**	**Mg**	**Al**	**K**	**P**	**S**	
T1	<0.1	9.8	4.6	12000	3000	41300	13300	609.7	7500	
T4	<0.1	8.4	12.6	16800	9200	64300	27500	639	12200	

Table 2. Physicochemical characteristics of two samples.

Sample	pH	Moisture content (%)	Organic carbon (g kg $^{-1}$)	Organic matter (g kg $^{-1}$)	CEC [a] (cmol kg $^{-1}$)
T1	7.33	62.25	13.17	22.7	10.44
T4	10.3	10	1.79	3.1	9.52

[a]CEC, Cation exchange capacity.

selected from each library. For RFLP and sequencing, the inserted fragments were amplified with the vector-specific T7 and SP6 primers. These unpurified PCR products were digested with two restriction endonucleases *AfaI* and *MspI* (are two enzymes sufficient for such purpose) (TaKaRa Corporation, Japan), incubated at 37°C for 3 h. The restricted fragments were separated by gel electrophoresis in 3.0% agarose with ethidium bromide staining and observed on UV illumination. RFLP patterns were identified and grouped, and representative clones were selected for nucleotide sequencing.

Phylogenetic analysis

Phylogenetic affiliations of the partial sequences were initially estimated using the program BLAST (basic alignment search tool) (Bond et al., 2000). Similarity of partial sequences was determined using ARB (a software environment for sequence data) (Strunk and Ludwig, 1997). The initial phylogenetic trees were based on all available sequences and were constructed by using the DNA distance program neighbor-joining with Felsenstein Correction in ARB (Smith et al., 1994). Based on the initial phylogenetic results, appropriate subsets of 16S rDNA sequences were selected and subjected to a final phylogenetic analysis with CLUSTAL X.

Statistical methods

The rarefaction analysis was performed with Sigma Plot software. An exponential model, $y = a \times [1 - \exp(-b \times x)]$, was used with Sigma Plot 8.0 non-linear regression software to fit the clone distribution data.

Nucleotide sequence accession numbers

54 Sequences have been submitted to GenBank with accession numbers from GQ487883 to GQ487936.

RESULTS AND DISCUSSION

Gochemical properties of samples

The differences of elements' concentrations in the two samples are shown in Table 1. Almost all the elements were detected and especially for heavy metals such as Cu, Pb, Cd, As and Mn, the concentrations of them in the first sample (T1) were much lower than that in the second sample (T4).

According to the National Environmental Quality Standard for Soils in China (GB 15618-1995), seen in Table 2, the content of Cu in first sample T1 exceeded the class III limitation (50 mg/kg for Cu), but in second sample T4, it exceeded the class III limitation (400 mg/kg for Cu). For heavy metal Cd, the concentration in sample T1 exceeded the class III limitation three times (0.6 mg/kg), but in sample T4, it was five times the class III limitation concentration.

Table 2 shows some physicochemical characteristics, such as pH, percentages of moisture content, organic carbon, organic matter and cation exchange capacity (CEC) of the samples. Sample T1 had a neutral pH, while sample T4 had a highly alkaline pH of 10.30. The moisture content, organic carbon, organic matter and CEC, which represent nutrient retention, increased significantly in sample T1 compared with that in sample T4. For example, the content of organic matter was 22.70 g/kg in sample T1, although it was lower than the usual range for arable land soil proposed by Porta et al. (2003), but it was more than seven times that in sample T4. The CEC value was higher in sample T1 than that in sample T4, which reflected larger capacity to retain both water

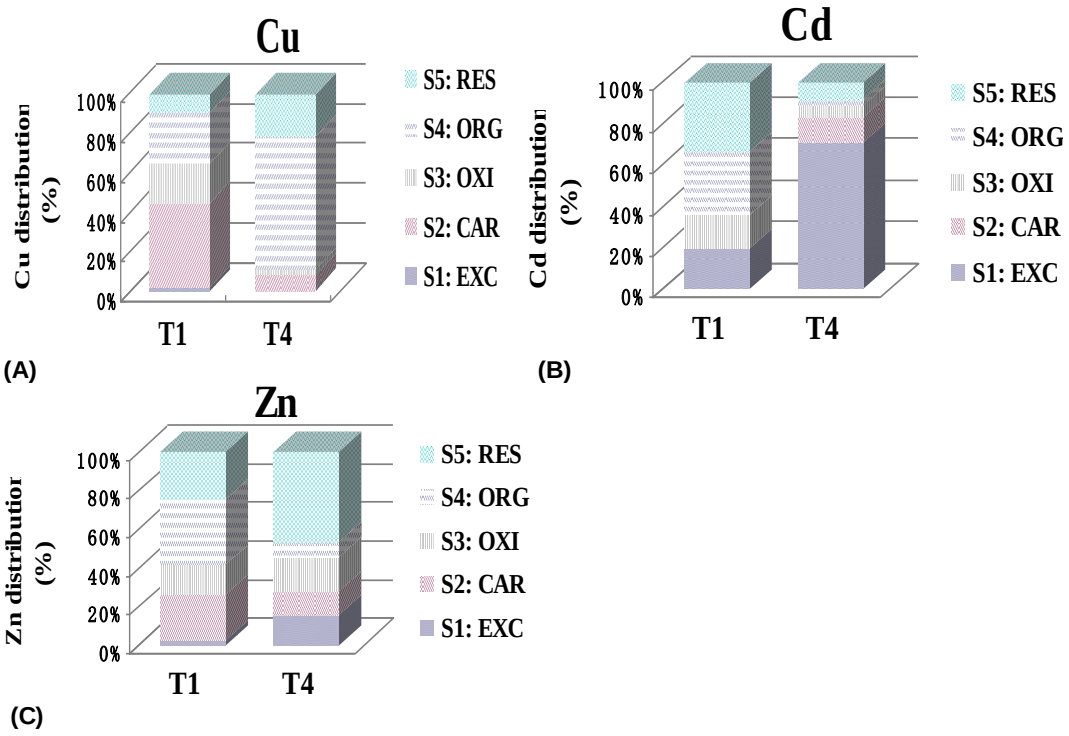

Figure 1. Distribution of the five chemical fractionations of Cu (A), Cd (B) and Zn (C) in the two samples. EXC, Exchangeable; CAR, bound to carbonates; OXI, amorphous iron-manganese oxides; ORG, organic-bound; RES, residual; T1, sample T1 obtained from the reclaimed 1# tailings dam of Dexing copper mine; T4, sample T4 obtained from the unreclaimed 4# tailings dam of Dexing copper mine.

and metals by sample T1 (Rodrıíguez et al., 2009).

Fractionation of Cu, Cd and Zn in soil samples

The toxicity and the mobility of heavy metals in soils depend not only on the total concentration, but also on their specific chemical forms (Rodrıíguez et al., 2009). Due to the high Cu and Cd concentrations analyzed in the samples, and the nature of copper mine, Cu and Cd were selected for chemical fractions study. Because Zn is one of the most concerned toxic heavy metals in China, it was also selected for the chemical speciation study. The metal fractions consist of exchangeable (EXC), bound to carbonates (CAR), amorphous iron-manganese oxides (OXI), organic-bound (ORG) and residual (RES) fraction were extracted from the bulk soil samples by Tessier's extraction method as presented in Table 1. The EXC fraction was considered as the bioavailability of metals. The percentages of each fraction of metals Cu, Cd and Zn are shown in Figure 1.

For Cu (Figure 1A), in sample T1, the dominant fraction was CAR fraction, which represented 42.8% of the total concentration of Cu in this sample whereas, in sample T4, the prevalent fraction was the ORG fraction, which represented 67.37% percentages of total concentration of Cu in the sample. Cu bioavailability (the EXC-Cu fraction) in sample T1 and sample T4 both was low, which occupied 2.1 and 0.85% in sample T1 and sample T4, respectively. This phenomenon may be due to copper's natural characteristcs. For copper, the organic-bound form was its main existence form due to the strong chelation, which could form dissolvable or undissolvable complex compound with many kinds of organic matter through complexation reaction. These complexation copper were very difficult to release (Wang and Wei, 1995).

For Cd (Figure 1B), sample T1 was dominated by the RES fraction (33.57%) and the ORG fraction (30.45%), but sample T4 was absolutely dominated by the EXC fracion (71.18%). Cd bioavailability (the EXC-Cd fraction) in sample T1 (19.55%) was much lower than that in sample T4 (71.18%). For Zn (Figure 1C), sample T1 was dominated by the ORG (34.22%), RES (24.13%) and CAR (24.08%) fractions, while sample T4 was mainly dominated by the RES fraction (46.83%). Zn bioavailability (the EXC-Zn fraction) in sample T1 (2.31%), was much lower than that in sample T4 (15.92%) too. Fellet et al. (2011) studied the application of pyrolyzed biomass as a phytostabilization technology to evaluate the amelioration of the mine tailings properties, and found that the bioavailability of Cd and Zn of the mine tailings decreased as the biochar content increased, which was consistent with the current results. The decreased bio-

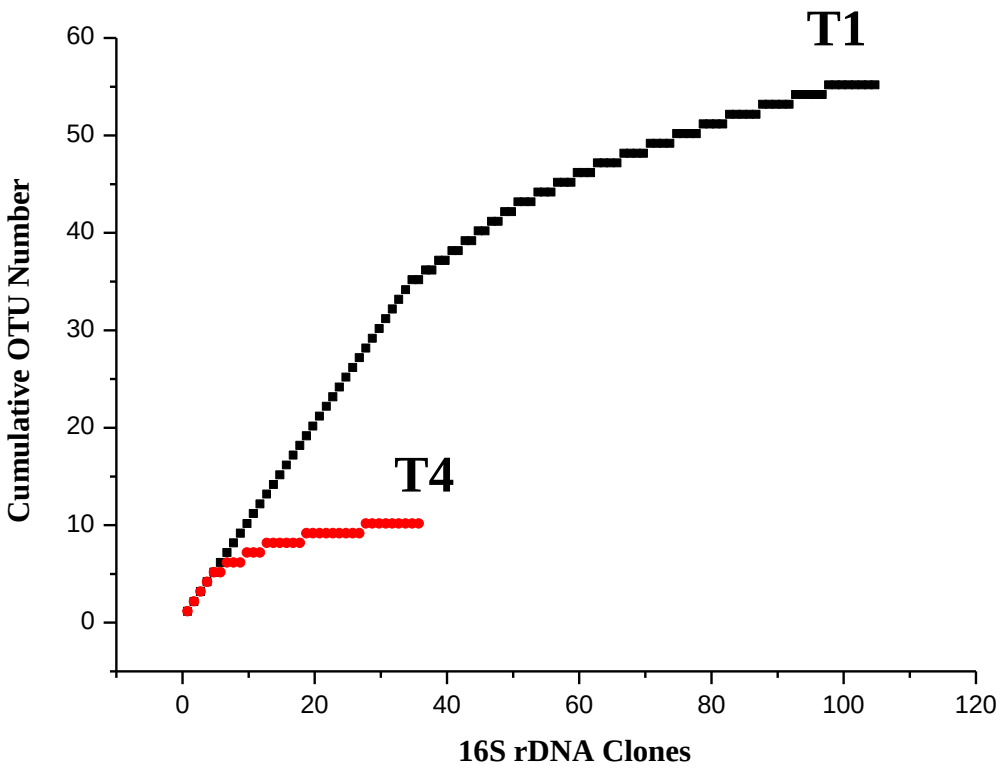

Figure 2. Evaluation of representative clones in the two samples. Clones containing bacterial 16S rDNA inserts. T1, Sample T1 obtained from the reclaimed 1# tailings dam of Dexing copper mine; T4, sample T4 obtained from the unreclaimed 4# tailings dam of Dexing copper mine.

availability of heavy metals Cd and Zn in sample T1 may have been from the reclamation, and may be further benefiting for the surviving microorganisms and plants growing in the site.

Analysis of bacterial 16S rDNA cloning libraries by RFLP

Bacterial 16S rDNA cloning libraries of these two samples were constructed. A total of 66 operational taxonomic units (OTUs) were obtained: 56 OTUs in sample T1, and 10 OTUs in sample T4. The rarefaction analysis was used in RFLP pattern analysis and results are shown in Figure 2. Non-linear regression in Figure 2 suggest that saturations were at 90 and 20 valid clones for sample T1 and T4, respectively for the construction of bacterial 16S rDNA cloning libraries, which suggested that number of valid clones tested in this experiment (105 valid clones tested for sample T1 and 36 valid clones tested for sample T4) were sufficient to detect the level of bacterial community diversity in the samples.

Phylogenetic analysis

To determine the phylogenetic diversity, representative 16S rDNA clones of OTUs that occurred more than once in cloning libraries, as well as representatives of unique OTUs, were fully sequenced. The phylogenetic analysis in samples was established with a bootstrap neighbor-joining method. The phylogenentic tree of bacterial 16S rDNA is shown in Figure 3. It could be seen that 12 phylogenetic divisions were represented in these two samples, including *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, α-*proteobacteria*, β-*proteobacteria*, *γ-proteobacteria*, *δ-proteobacteria*, *Planctomycetes*, *Chlorobi*, *Chloroflexi*, *Nitrospira* and *Firmicutes*. The detailed distribution of each division in the two samples is shown in Figure 4.

Results in Figures 3 and 4 show significant difference in the structure of microbial community composition in the two samples. In sample T1 (Figure 4A), communities were dominated by *Planctomycetes* (20% of the total 105 clones), α-*proteobacteria* (16%), *Chloroflexi* (12%) and *Acidobacteria* (10%), and the remaining 8 divisions ranged from 3 to 8%. In sample T4 (Figure 4B), there were only four phyla presented, including γ-*proteobacteria* (86% of the total 36 clones), β-*proteobacteria* (8%), *Bacteroidetes* (3%), and *Planctomycetes* (3%). Compared with the phylotypes presented in sample T4, percentage of *γ-proteobacteria* significantly decreased in sample T1, while the percentage of divisions *Planctomycetes* and *Bacteroidetes* increased, and divisions of α-*proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Actinobacteria*, *δ-proteobacteria*, *Chlorobi*, *Nitrospira* and *Firmicutes*

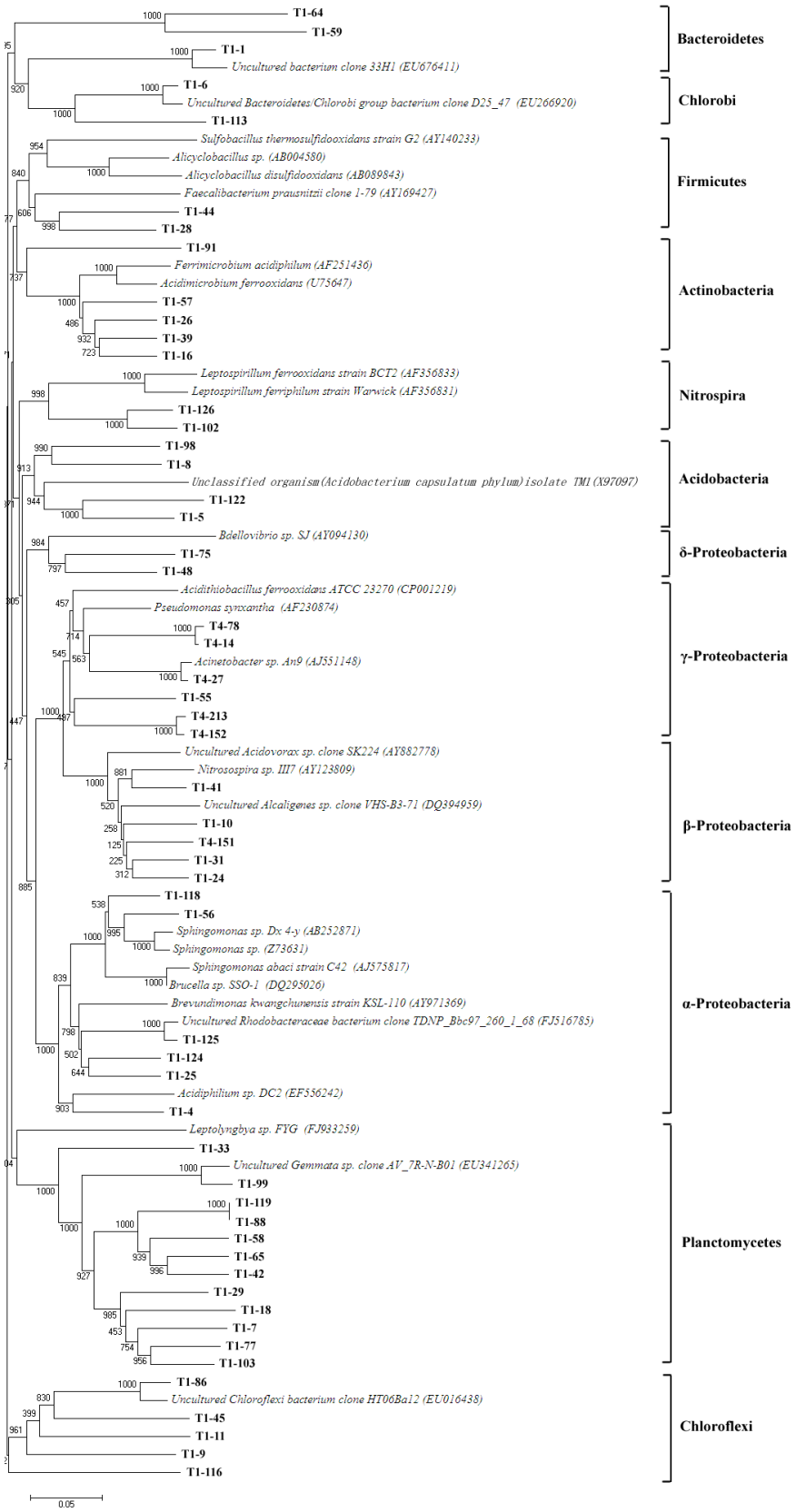

Figure 3. Phylogenentic tree based on comparative analysis of bacterial 16S rDNA sequence data and their relatives. The sequences obtained in this study are indicated in bold.

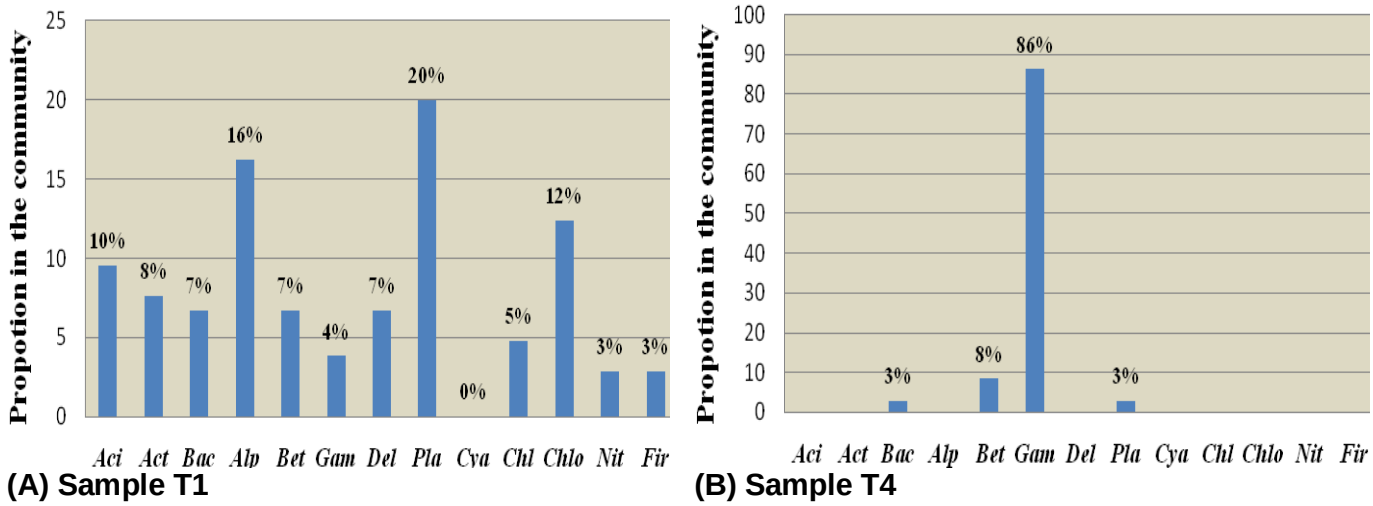

Figure 4. Profile of bacterial divisions in the two samples. **A,** sample T1. **B,** sample T4; Aci, *Acidobacteria*; Act, *Actinobacteria*; Bac, *Bacteroidetes*; Alp, α-*proteobacteria*; Bet, β-*proteobacteria*; Gam, *γ-proteobacteria*; Del, *δ-proteobacteria*; Pla, *Planctomycetes*; Chl, *Chlorobi*; Chlo, *Chloroflexi*; Nit, *Nitrospira*; Fir, *Firmicutes*.

appeared in the sample T1.

It could be seen that the reclaimed tailings sample had obviously higher diversity of microorganisms than that in the unreclaimed tailings sample. Monica et al. (2008) compared the bacterial communities presented in acidic lead-zinc mine tailing samples with a control soil sample, they revealed that *γ-proteobacteria* and *Firmicutes* were dominant phyla in tailing samples, while in control soil sample, *γ-proteobacteria* phylotype decreased, *Acidobacteria*, *Actinobacteria*, α-*proteobacteria*, β-*proteobacteria* increased, and *δ-proteobacteria* appeared newly. It indicated that reclamation and revegetation resulted in increasing diversity of microbial community in reclaimed sample. Meanwhile, the phylogenetic diversity observed in a typical of an average healthy soil was defined by Janssen (2006).

In a survey of 32 clone libraries from a broad range of soils, Janssen found that average soil communities were dominated by *Proteobacteria* (39%, including 19% α-*proteobacteria*, 10% β-*proteobacteria*, and 8% *γ-proteobacteria*), followed by *Acidobacteria* (20%), and *Actinobacteria* (13%). The remaining phyla representing 2 to 7% of the clones were *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, and *Gemmatimonadetes* (Janssen, 2006; Monica et al., 2008). In the current study, the sample T1 was more similarly dominated by *Proteobacteria* (34%, including 16% α-*proteobacteria*, 7% β-*proteobacteria*, 4% *γ-proteobacteria,* and 7% *δ-proteobacteria*), followed by *Planctomycetes* (20%), *Chloroflexi* (12%), *Acidobacteria* (10%), and *Actinobacteria* (8%) (Figure 4A).

Out of Janssen's five dominant phyla, only the β-*proteobacteria* and *γ-proteobacteria* were represented by clones in sample T4 bacterial community (Figure 4B). These results may suggest that the reclaimed tailings sample T1 was able to sustain more phyla associated with a healthier soil. The changes may mainly be due to the improved soil conditions, such as neutral pH value, increased moisture content, organic carbon, organic matter and CEC, and decreased heavy metal bioavailability after the reclamation in sample T1.

Compared with the composition of microbial communities in these two samples, it could be proposed that inventories of bacterial populations such as *Planctomycetes, Chloroflexi,* α-*proteobacteria*, *Acidobacteria* and *Actinobacteria*, dominated in reclaimed tailings sample that are capable of sustaining plant growth, but absent from unreclaimed tailings sample, which may provide information regarding the potentiality of those organisms as bioindicator for evaluation of status of reclamation of contaminated soil.

The *planctomycetes* are a group of budding bacteria that lack peptidoglycan and possess membrane-bound intracellular compartments (Fuerst, 2005). Most isolates of this phylum are from aquatic sources. Isolates from soil, including a few members of the WPS-1 lineage, was reported by Davis et al. (2005), but these do not represent the full phylogenetic breadth suggested by the sequences detected in soils. In the current study, 20% of sample T1 clones and 3% of sample T4 clones were affiliated with the *Planctomycetes* division. The representative sequence T1-99 representing four clones from first sample and one clone from second tailings sample, had 95% similarity to uncultured *Gemmata* sp. Clone AV_7R-N-B01 fell into the *Planctomycetes* division (Figure 3).

The phylum *Chloroflexi* consists of perhaps eight candidate classes (Rappe´ and Giovannoni, 2003). Only one isolate from soil has been reported. It is a filamentous aerobic heterotroph, but no conclusions can be drawn yet about the general properties of soil *Chloroflexi*

(Jassen, 2006). In this study, five representative sequences grouped with the uncultured *Chloroflexi* bacterium clone HT06Ba12 in the *Chloroflexi* division were detected only in the sample T1 bacterial clone library.

The division of α-*proteobacteria* was formed by representative sequences T1-56, T1-118, T1-125, T1-124, T1-25 and T1-4, which represented 16% of the total clones from sample T1. They were closely related to the drinking water bacterium MB13 (with similarity 99%), *Sphingomonadaceae* bacterium CBFR-1 (similarity 96%), uncultured *Rhodobacteraceae* bacterium clone TDNP_Bbc97_260_1_68 (similarity 98%), uncultured bacterium clone mv13.4 (similarity 99%), uncultured bacterium clone CBNH_072904_C36 (similarity 99%) and Bacterium Ellin331 (similarity 99%), respectively in the database, and were mainly grouped with *Sphingomons* sp. and *Acidiphilium* sp. in the α-*proteobacteria* division (Figure 3). *Sphingomonas* is a novel bioresource and widely spread in various aquatic and terrestrial environments.

Yabuuchi et al. (1990) described the characteristics of this genus for the first time. Some strains of *Sphingomonas* species have been isolated from heavy metal polluted soils around industry factories. Sun et al. (2009) isolated copper tolerant strains *Sphingomonas* sp. (JM14, YM22 and YM12) from copper contaminated soils. Our previous study isolated a *Sphingomonas* sp. strain DX-T3-03 that had high tolerance to Zn (Xie et al., 2010). *Acidiphilium* sp. is an heterotrophic microorganism that may readily be isolated from most acidic mine water and different mine tailings, such as *Acidiphilium* isolates CH3 isolated from the La Andina copper mine tailing, Chile (Diaby et al., 2007; Monica et al., 2008), and *Acidiphilium* sp. PK40 isolated from acid streamers from abandoned copper mines in north Wales, United Kingdom (Hallberg et al., 2006; Monica et al., 2008). In our study, all the sequences related to these species were found only in sample T1, not in sample T4.

The *Acidobacteria* are characterized as moderately acidophilic heterotrophs, preferring a pH of 3 to 6. Recent research has shown that the capacity to reduce iron is also widespread among these bacteria (Rowe et al., 2007). In this study, four representative sequences: T1-98, T1-8, T1-122 and T1-5 were grouped with the unclassified organism *Acidobacterium capsulatum* phylum isolate, and fell into the *Acidobacteria* division, which were only found in sample T1 (Figure 3).

In the *Actinobacteria* division, representative sequences T1-57, T1-26, T1-39 and T1-16, representing 5.7% of the total clones from sample T1, were grouped with *Acidimicrobium ferrooxidans* and *Ferrimicrobium acidiphilum* species (Figure 3). The *A. ferrooxidans* species such as *A. ferrooxidans* AMD clone BA46 were identified by Bond et al. (2000) and clone ASL4 was been identified by Baker and Banfield (2003) from the Richmond mine in Iron Mountain, CA. The *F. acidiphilum* (AF251436) was reported as a heterotrophic iron oxidizer (monica et al., 2008). In the current study, all the grouped clones were only detected in sample T1.

Conclusion

The reclaimed tailings sample had higher diversity of microbiota and had more reasonable structure of microbial community composition associated with healthy soil than that in unreclaimed tailings sample T4. The inventtories of bacterial populations dominated in reclaimed sample such as *Planctomycetes, Chloroflexi,* α-*proteobacteria*, *Acidobacteria* and *Actinobacteria*, may be set as potential bioindicators for reclamation evaluation. The differences of microbial community composition between reclaimed and unreclaimed samples may mainly be attributed to the improved soil conditions by reclamation. Further investigation which focused on impacts of soil conditions and heavy metals bioavailability on structure of bacterial community has been carried out.

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REFERENCES

Abollino O, Aceto M, Malandrino M, Mentasti E, Sarzanini C, Petrella F (2002). Heavy metals in agricultural soils from Piedmont, Italy. Distribution, speciation and chemometric data treatment. Chemosphere 49:545-557.

Baker BJ, Banfield JF (2003). Microbial communities in acid mine drainage. FEMS. Microbiol. Ecol. 44:139-152.

Bartels JM (1996). Methods of Soil Analysis. Part III. Chemical Methods. In: Sparks, D.L. (Ed.), Soil Science Society of America. Madison, Wisconsin, USA, pp.1201-1230.

Bond PL, Smriga SP, Banfield JF (2000). Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. Appl. Environ. Microb. 66:3842-3849.

Chen HM, Zheng CR, Zhou DM, Tu C, Gao L (2005). Changes in soil fertility and extractable heavy metals in Dexing copper mine tailing pool after revegetation. Acta pedologica sinica. 42:29-36.

Davis KER, Joseph SJ, Janssen PH (2005). Effects of growth medium, inoculum size, and incubation time on the culturability and isolation of soil bacteria. Appl. Environ. Microb. 71:826-834.

De La Iglesia R, Castro D, Ginocchio R, Van Der Lelie D, Gonzalez B (2006). Factors influencing the composition of bacterial communities found at abandoned copper-tailings dumps. J. Appl. Microbiol. 100:537-544.

Diaby N, Dold B, Pfeifer H, Holliger C, Johnson DB, Hallberg KB (2007). Microbial communities in a porphyry copper tailings impoundment and their impact on the geochemical dynamics of the mine waste. Environ. Microbiol. 9:298-307.

Fabienne G, Antonis C, Hauke H (2003). Comparative 16S rDNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal

contaminated bulk and rhizosphere soil. Environ. Microbiol. 10:896-907.
Fellet G, Marchiol L, Delle Vedove G, Peressotti A (2011). Application of biochar on mine tailings: Effects and perspectives for land reclamation. Chemosphere 83:1262-1267.
Fuerst JA (2005). Intracellular compartmentation in planctomycetes. Annu. Rev. Microbiol. 59:299-328.
Hallberg KB, Coupland K, Kimura S, Johnson DB (2006). Macroscopic streamer growths in acidic, metal-rich mine waters in North Wales consist of novel and remarkably simple bacterial communities. Appl. Environ. Microb. 72:2022-2030.
Lane DJ (1991). 16S/23S rDNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. John Wiley & Sons, Chichester, England, pp.115-175.
Li MS (2006). Ecological restoration of mineland with particular reference to the metalliferous mine wasteland in China: a review of research and practice. Sci. Total Environ. 357:38-53.
Li YT, Becquer T, Dai J, Quantin C, Benedetti M (2009). Ion activity and distribution of heavy metals in acid mine drainage polluted subtropical soils. Environ. Pollut. 157:1249-1257.
Londry K, Sherriff B (2005). Comparison of microbial biomass, biodiversity, and biogeochemistry in three contrasting gold mine tailings deposit. Geomicrobiol. J. 22:237-247.
Mummey DL, Stahl PD, Buyer JS (2002). Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. Appl. Soil Ecol. 21:251-259.
Munshower FF (1994). Practical handbook of disturbed land revegetation. Lewis Publishing, Boca Raton, FL.
Monica OM, Julia WN, Raina MM (2008). Characterization of a Bacterial Community in an Abandoned Semiarid Lead-Zinc Mine Tailing Site. Appl. Environ. Microb. 74:3899-3907.
Moynahan OS, Zabinski CA, Gannon JE (2002). Microbial community structure and carbon-utilization diversity in a mine tailings revegetation study. Restor. Ecol. 10:77-87.
Pawloska TE, Chaney RL, Chin M, Charvat I (2000). Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. Appl. Environ. Microb. 66:2526-2530.
Porta J, López-Acevedo M, Roquero C (2003). Edafología para la agricultura y el medio ambiente. Ediciones Mundi-Prensa. p.849.
Rappe´ MS, Giovannoni SJ (2003). The uncultured microbial majority. Annu. Rev. Microbiol. 57:369-394.
Rodrıíguez L, Ruiz E, Alonso-Azcárate J, Rincón J (2009). Heavy metal distribution and chemical speciation in tailings and soils around a Pb–Zn mine in Spain. J. Environ. Manage. 90:1106-1116.
Rowe OF, Sa´nchez-Espan˜a J, Hallberg KB, Johnson DB (2007). Microbial communities and geochemical dynamics in an extremely acidic, metal-rich stream at an abandoned sulfide mine (Huelva, Spain) underpinned by two functional primary production systems. Environ. Microbiol. 9:1761-1771.
Sheoran AS, Sheoran V (2006). Heavy metal removal mechanism of acid mine drainage in wetlands: a critical review. Miner. Eng. 19: 105-116.
Smith SW, Overbeek R, Woese CR (1994). The genetic data environment: an expandable GUI for multiple sequence analysis. Comput. Appl. Biosci. 10: 671-675.
Strunk O, Ludwig W (1997). ARBda Software Environment for Sequence Data. Department of Microbiology. Technical University of Munich, Munich, Germany.
Sun LN, Zhang YF, He LY (2009). Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-tolerant plant species on copper mine wasteland. Bioresour. Technol. 101:501-509.
Tessier A, Campbell P, Bisson M (1979). Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51:844-851.
Tessier A, Campbell P, Bisson M (1980). Trace metal speciation in the Yamaska and St. Francois Rivers (Quebec). Can. J. Earth Sci. 17:90-105.
Wang YP, Shi JY, Lin Q, Chen XC, Chen YX (2007). Heavy metal availability and impact on activity of soil microorganisms along a Cu/Zn contamination gradient. J. Environ. Sci. 19:848-853.
Wang Y, Wei FS (1995). Soil environmental element chemistry. Peking, People´s Republic of China: Chinese Environmental Science Press (in Chinese).
Xie XH, Fu J, Wang HP, Liu JS (2010). Heavy metal resistance by two bacteria strains isolated from a copper mine tailing in China. Afr. J. Biotechnol. 9(26):4056-4066.
Yabuuchi E, Yano I, Oyaizu H, Hashimoto H, Ezaki T, Yamamoto H (1990). Proposals of Sphingomonas paucimobilis gen nov. and comb nov., Sphingomonas parapaucimobilis sp.nov., Sphingomonas yanoikuyae sp.nov., Sphingomonas adhaesiva sp. nov., Sphingomonas capsulatecomb nov. and two genospecies of genus Sphingomonas. Microbiol. Immunol. 2:99-119.
Zhou J, Bruns MA, Tiedje JM (1996). DNA recovery from soils of diverse composition. Appl. Environ. Microb. 62:316-322.