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 phyto-stabilization 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 un-reclaimed 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 physico-chemical 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 km². 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 dam slope of the 1# tailings site, which has the biggest tailings pool dam, was vegetated for nearly 20 years. The other tailings sites, which has the biggest tailings pool dam and dam in Asia, is still under work and will need reclamation. 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.

DNA extraction and purification

Extraction 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 Miniprep DNA purification system (Promega Corporation, USA) and quantified by ethidium 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- AGAGTTTGATCCTGCTGAG-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 isolated 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.

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Table 1. Elements’ concentrations in the two samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zn (mg/kg)</th>
<th>Fe (mg/kg)</th>
<th>Cu (mg/kg)</th>
<th>Pb (mg/kg)</th>
<th>Cd (mg/kg)</th>
<th>Cr (mg/kg)</th>
<th>Hg (mg/kg)</th>
<th>As (mg/kg)</th>
<th>Mn (mg/kg)</th>
<th>Ag (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>22.8</td>
<td>25000</td>
<td>106.2</td>
<td>17.4</td>
<td>1.7</td>
<td>40.1</td>
<td>0.04</td>
<td>8.8</td>
<td>186.3</td>
<td>0.7</td>
</tr>
<tr>
<td>T4</td>
<td>44.6</td>
<td>31600</td>
<td>452.4</td>
<td>37.7</td>
<td>2.7</td>
<td>45.8</td>
<td>0.05</td>
<td>11.8</td>
<td>373.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 2. Physicochemical characteristics of two samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Moisture content (%)</th>
<th>Organic carbon (g kg(^{-1}))</th>
<th>Organic matter (g kg(^{-1}))</th>
<th>CEC (^{a}) (cmol kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>7.33</td>
<td>62.25</td>
<td>13.17</td>
<td>22.7</td>
<td>10.44</td>
</tr>
<tr>
<td>T4</td>
<td>10.3</td>
<td>10</td>
<td>1.79</td>
<td>3.1</td>
<td>9.52</td>
</tr>
</tbody>
</table>

\(^{a}\)CEC, Cation exchange capacity.

RESULTS AND DISCUSSION

Geochemical 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 and nutrients.
and metals by sample T1 (Rodríguez 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 (Rodriguez 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 characteristics. 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 fraction (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 1. Distribution of the five chemical fractions 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.
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 phylogenetic 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 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.
Figure 3. Phylogenetic tree based on comparative analysis of bacterial 16S rDNA sequence data and their relatives. The sequences obtained in this study are indicated in bold.
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. Monaco 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 bioindicators 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 HT06B4a12 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 CBF1 (99% similarity), uncultured Rhodobacteraceae bacterium clone TDNP_Bbc97_260_1_68 (98%), uncultured bacterium clone mv13.4 (99%), uncultured bacterium clone CBNH_072904_C36 (99%) and Bacterium Ellin331 (99%), respectively in the database, and were mainly grouped with Sphingomonas 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 ferroxidans and Ferrimicrobium acidiphilum species (Figure 3). The A. ferroxidans species such as A. ferroxidans 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 inventories 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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