Microbial Ecology

Bacterial Diversity in Mine Tailings Compared by Cultivation and Cultivation-independent Methods and their Resistance to Lead and Cadmium

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Received: 16 December 2006 / Accepted: 6 February 2007 / Online publication: 2 March 2007

Abstract

To examine bacterial community composition in rhizosphere of plants colonizing on mine tailings and phylogenetic differences between subcommunities resistant to different metals, we constructed four clone libraries of 16S rDNA sequences. One was amplified directly from tailing microbial DNA (named as Ci library) and three from cultures on the plates containing of 0.5 mM $CdCl_2$ (Cd library), 2 mM Pb $(NO_3)_2$ (Pb library), and without any metals (Cw library). In total, nine bacterial divisions and two unclassified groups were identified from 352 clones of these libraries. Ci clones covered eight divisions, whereas all cultivable clones only covered four divisions. Thus, Ci library provided more phylogenetic diversity than cultivable libraries. However, the microbes represented by the cultivable clones were more similar to previously described bacteria than those represented by Ci clones. All Ci clones were not found in three cultivable libraries. Cd library were exclusively Gram-negative bacteria of *Acinetobacter*, *Ralstonia*, *Comamonas*, and *Chryseobacterium*. Meanwhile, dominant Gram-positive bacteria in Pb library, *Paenibacillus* and *Bacillus*, were also not found in Cd library. Our data indicate that phylogenetic structure was very different from those in acid mine drainage. Meanwhile, tailings harbored phylogenetically distinct subcommunities resistant to Pb and Cd.

Introduction

Tailings adversely affect the quality of surrounding soils and aquifers [6, 20]. Remediation of tailing ecosystem, either by phytoextraction [19] or by direct microbial bioremediation *in situ* [5, 13], greatly depends on the information of microbial ecology because microorganisms have been well known to change metal speciation and mobility and, consequently, affect the performance of plants in such environments [13, 19]. Therefore, adjustment of the physical and chemical parameters into a range more conducive to microbial activity should be an effective support for biological recovery [1].

However, until now, most microbial information in tailing ecosystems comes from acid mine drainage (AMD) [20, 31]. Moreover, previous investigations have mainly demonstrated metabolic activity [12, 31]. Microbial community structure has been described only in a few categories of tailings, such as sulfidic mine waste and uranium tailings, by culture-based methods [27, 31]. Therefore, a comprehensive microbial composition in mine tailings still remains to be uncovered due to the great chemical and physical heterogeneity of tailings over the world [8].

In addition, stress of heavy metals is well-known to shift a native bacterial community to a composition in which tolerant organisms become numerously dominant [7]. Currently, most of our knowledge about this resulting community is also confined to the changes of their activity [12] and phospholipids fatty acid (PLFA) values [25]. Although PLFA composition and quantity, in some cases, could be correlated to specific groups of microbes, the PLFA profile does not give an actual species composition [18].

More importantly, metal bioavailability is known to have apparently dramatic variations from site to site in soils [3], and the small-scale heterogeneity of metal concentrations and partitioning may result partially from the heterogeneous distribution of metal sorbents, i.e., organic C [34]. Similarly, in reality, microbial distribu-

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tion is spatially extremely uneven in soils. They range in soil from single cells to microcolonies to biofilms [15]. Obviously, the frequency of pollutant molecule encounters by soil microbes is a function of cell distribution pattern, and subsequently, an undisturbed site with multiple heavy metals would harbor both tolerant species adaptive to different metal pools and sensitive species colonizing in a microniche without metals. Therefore, above overall changes of community structure in contaminated sites may simplify the relationships between soil microbes and pollutants.

In the present study, our objective was to examine microbial community composition in mine tailings contaminated with high concentrations of Pb and Cd through a cultivation-independent method. Furthermore, we also performed experiments to evaluate if it is similar in phylogenetic composition between communities resistant to different metals through a cultivation-dependent method. First, we used restriction fragment length polymorphism (RFLP) of 16S rDNA sequences to define phylotypes. Then, partial sequences obtained from representatives of the RFLP patterns were used to analyze phylogenetic diversity and compare the phylotype richness and composition of four clone libraries.

Materials and Methods

Sampling of Tailings. Samples were collected from abandoned mine sites (tailing pile B) [36] in northern Yunnan Province, China. Tailings were piled in the open air, sparsely covered with grass and shrub. Tailings were collected from rhizosphere of plants in July 2005. These plants included *Clinopodium repens*, *Incarvillea arguta*, *Carex fluviatilis*, *Lotus corniculatus*, *Ficus tikoua*, *Hypericum beanii*, *Argyranthemum apiacea*, *Eragrostis ferruginea* var. *yunnanensis*, *Oplismenus compsitus*, *Silene viscidula*, *Arenaria serpyllifolia*, *Anemone vitifolia*, *Diospyros mollifolia*, *Deyuxia scabrescens*, and *Oxyria sinensis*. For each plant, 10–20-g subsamples were collected from rhizosphere and then pooled to create a single composite sample. The composite sample was mixed well and immediately transported back to laboratory and frozen at −70°C. Metal contents were determined by atomic absorption spectrophotometer. The pH was determined by adding 5 ml of distilled water to 5 g of air-dried tailings and measuring the pH of the tailing slurry.

Extraction of DNA. Total microbial DNA was directly extracted from 100-mg tailings by using SoilMaster DNA Extraction Kit (Epicentre, Madison, WI, USA) following the product protocol. To obtain cultivable and heavy metal-resistant bacteria, 10 g of tailings was suspended with 90 ml of sterile water and rotated for 20 min. Appropriate cell suspensions were spread onto TY solid plates (1000 ml distilled water containing 3 g of yeast extract, 5 g of peptone, 3 g of tryptone, and 15 g of agar, pH 7.2–7.4) with 0.5 mM $CdCl_2$, 2 mM $Pb(NO_3)_2$, and without any metals. After incubation at 28°C for 3 days, colonies were observed and counted. The data were expressed as colony-forming units (CFUs) per gram dry weight of each sample. Then, cells of three 10^{-1} dilution plates (about 10^8 colonies each plate) from each treatment were washed off with sterile water and pooled. Cells were further washed twice and titrated. Genomic DNA of about 10^8 cells from each treatment was extracted using the method described above.

Construction of 16S rDNA Libraries. 16S rRNA gene was amplified by using the conserved primer pairs F968 and R1401 [11, 23]. Polymerase chain reaction (PCR) products approximately 400 bp long were ligated into the pMD18-T Simple Vector (TakaRa, Dalian, China) and transformed into competent *Escherichia coli* HB101 cells. Plasmid clones were identified based on blue–white screening. Approximately 100 clones containing inserts of the correct size were stored in 20% glycerol at −20°C for each treatment and constructed into four clone libraries. These libraries were designated as cultivation-independent (Ci), Cd cultivation (Cd), Pb cultivation (Pb), or cultivation without metals (Cw).

RFLP Analysis. Target 16S rDNA fragments of clones were amplified directly from fresh cell suspension resuscitated from above glycerol stock by using primer M13. After PCR amplification, 2 μl of target fragments from each clone was digested separately with 1.0 U of *Rsa*I, *Bsu*RI, and *Hinf*I (Bio Basic, Markham, Canada) in 5-μl reaction mixtures [11]. Digests were electrophoresed in 3% gels with ethidium bromide and then photographed by using GIS system (Tanon, Shanghai, China). Patterns of DNA fragment size were checked manually, and each phylotype was defined as a group of clones that had indistinguishable enzyme restriction pattern.

Phylogenetic Analysis. Partial 16S rDNA sequences from one representative clone of each phylotype were sequenced commercially. DNA sequences were checked and edited carefully by using SeqMan and EditSeq software (DNAStar, Madison, WI, USA). Then, all sequences were analyzed for the presence of chimeras by using the Chimera Check program [Ribosomal Database Project (RDP), version 8.1].

To construct a phylogenetic tree, the closest relatives were searched and downloaded by using the Seqmatch (version 3.0) program of RDP. Sequence similarity was represented by $S_{\_ab}$ value, which was defined as the number of (unique) oligomers shared between our

sequence and a given RDP sequence divided by the lowest number of unique oligos in either of the two sequences (http://rdp8.cme.msu.edu/docs/seq_match_doc.html). The phylogenetic tree was constructed by using the PHYLIP package (version 3.65) with Jukes–Cantor evolutionary model and neighbor-joining method. Bootstrap analysis was carried out for 100 replicates.

To determine whether microbial community represented by each library was different, all clones from each phylotype were viewed as the repeats of that phylotype's representative sequence. Subsequently, homologous coverage curves, $C_x(D)$, and heterologous coverage curves, $C_{xy}(D)$, of these deduced sequences were calculated by the integral form by using ∫-LIBSHUFF program [29]. Rarefaction curves were calculated by using the DOTUR program with the furthest neighbor assignment algorithm [28]. Sampling coverage was estimated with Good formula [14].

Nucleotide Sequence Accession Numbers. The nucleotide sequences determined in this study have been deposited in GenBank under accession numbers DQ487875 to DQ487955.

Results

Tailing Chemistry and Bacterial Number. Only Pb and Cd concentrations and pH values of tailings were determined. Total and soluble Pb concentrations were 5883 and 130 mg kg^{-1} of tailings, respectively. Total and soluble Cd concentrations were relatively low, only 80.2 and 16.5 mg kg^{-1} of tailings, respectively. Tailings showed a slight basic pH value (7.85) and no acidification occurred. The culturable bacterial population size was 1.45×10^6 CFU g^{-1} of tailings when cells were cultured on TY medium without any heavy metals. Culturable bacteria resistant to 2 mmol l^{-1} of $Pb(NO_3)_2$ and to 0.5 mmol l^{-1} of $CdCl_2$ were 7.5 and 1.3×10^5 CFU g^{-1} of tailings, respectively.

RFLP Phylotypes. To determine bacterial community composition and whether it is similar to phylogenetic position between bacterial communities grown under the stress of 2 mmol l^{-1} of $Pb(NO_3)_2$ and to 0.5 mmol l^{-1} of $CdCl_2$, four clone libraries were constructed and RFLP analysis was carried out. In total, 87 phylotypes were identified from 371 clones based on their three-enzyme-digested patterns of 16S rDNA fragments. Typically, an approximately 400-bp 16S rDNA sequence of one representative clone from each phylotype was used to evaluate the phylogenetic diversity. Excluding eight possible chimeras, represented by 19 clones, from further analysis, we finally obtained 79 phylotypes, represented by 352 clones. Thirty-three phylotypes were identified from 98 Ci clones, whereas 46 phylotypes occurred among 254 clones from Cd, Pb, and Cw libraries. Relatively, the Ci library had 80% of sample coverage, lower than that of three cultivable libraries, all of them more than 90% (Table 1).

If only isolate sequences (total 74,843 sequences) were included in the RDP search, of 33 Ci phylotypes, only five, represented by seven clones, showed $S_{\_ab}$ values greater than 0.80 and found the nearest neighbor previously named and cultivated. On average, $S_{\_ab}$ value was low, ranging from 0.41 to 0.97, with a median value, 0.61. However, with the exception of K16, all phylotypes from three cultivable libraries found the nearest relatives and showed high $S_{\_ab}$ values ranging from 0.83 to 1.00, with a median value of 0.97. Therefore, the microbes represented by the cultivable clones were more similar to previously identified bacteria than those represented by Ci clones.

Phylogenetic Diversity. Sequences with the highest similarity to each representative phylotype were downloaded from the RDP database when both environmental and isolate sequences were included in RDP search (total 210,059 sequences). Then, they were used to construct phylogenetic tree with our sequences. These phylotypes belonged to nine divisions and two unclassified groups (Fig. 1, Table 2). With the exception that B53 occurred in both the Cd and Cw libraries and K7 occurred in both the Pb and Cw libraries, all phylotypes occurred only in one library.

Bacterial diversity was the widest in the Ci library, covering eight divisions and two unclassified groups. The most numerical clones were *Acidobacteria*, including 11 phylotypes represented by 64 clones. These phylotypes were divided into three clusters. The secondarily numer-

Table 1. Coverage of RFLP phylotypes in 16S rDNA clone libraries

Parameter	*Ci*	*Cd*	*Pb*	*Cw*	*Total*
Total no. of patterns	33 (98)	12 (75)	15 (77)	19 (102)	79 (352)
No. of unique patterns[a]	33 (98)	11 (61)	14 (75)	17 (90)	75 (324)
% of unique patterns	100	91.7	93.3	89.5	94.9
No. of singletons[b]	18	6	4	10	38
% of sample coverage[c]	81.6	91.8	94.8	90.2	89.3

The values in parentheses are the numbers of clones.
[a]Patterns that were found in only one clone library.
[b]Phylotypes that were represented by only a single clone in a clone library.
[c]Good coverage [14].

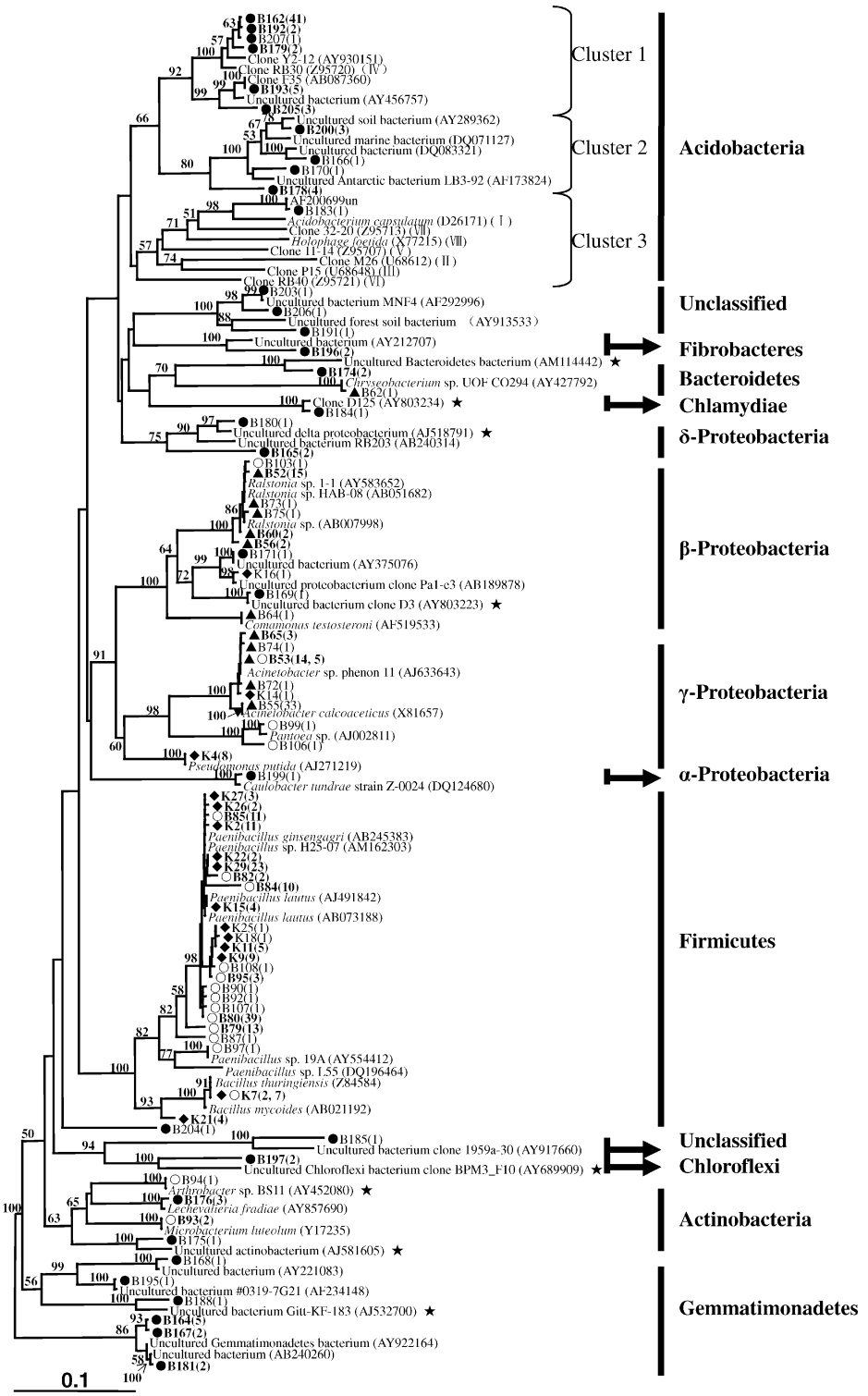

Figure 1. Phylogenetic relationship of representative 16S rRNA gene sequences from our tailings affiliated with relatives from RDP database. Phylotypes with *white circles*, *black circles*, *diamonds*, and *triangles* represent sequences obtained from Cw, Ci, Pb, and Cd libraries, respectively. Clones printed in *bold* are those with more than one time of occurrence in the clone library, and their occurrence times are indicated in parenthesis. *Black star* represents reference sequences obtained previously from heavy metal-contaminated environments. *Roman numbers* in parentheses represent subdivisions 1 to 8 of *Acidobacteria* [16]. Bootstrap values (100 replications) of equal to or >50% are indicated at *branch nodes*. GenBank accession numbers of reference sequences are shown in parentheses. The *scale bar* represents 10% estimated sequence divergence.

ical clones were *Gemmatimonadetes*, including six phylotypes represented by 12 clones. Other phylotypes belonged to *Actinobacteria*, *Bacteroidetes*, *Chlamydiae*, *Chloroflexi*, *Fibrobacteres*, and *Proteobacteria*. *Proteobacteria* contained subdivision *Alphaproteobacteria*, *Betaproteobacteria*, and *Deltaproteobacteria*. However, these divisions belonged to the minority and were only represented by one or two clones (Table 2).

One hundred and two Cw clones covered *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. Dominant sequences were *Firmicutes*, including 13 phylotypes represented by 91 clones. All sequences were highly

Table 2. Comparison of distribution of phylogenetic groups of bacteria detected in tailings by different methods

	Phylotype abundance			
Phylum	*Ci*	*Pb*	*Cd*	*Cw*
Acidobacteria	11 (64)			
Actinobacteria	2 (4)			2 (3)
Bacteroidetes	1 (2)		1 (1)	
Chlamydiae	1 (1)			
Chloroflexi	1 (2)			
Firmicutes		12 (67)		13 (91)
Gemmatimonadetes	6 (12)			
Fibrobacteres	1 (2)			
Proteobacteria				
Alphaproteobacteria	1 (1)			
Betaproteobacteria	2 (2)	1 (1)	6 (22)	1 (1)
Gammaproteobacteria		2 (9)	5 (52)	3 (7)
Deltaproteobacteria	2 (3)			
Unclassified	5 (5)			
Total	33 (98)	15 (77)	12 (75)	19 (102)

The values in parentheses are the number of clones.

related to members of genus *Paenibacillus*. Two single-sequence phylotypes were genus *Pantoea*. One single-sequence phylotype, B103, was related to *Ralstonia*. In addition, phylotype B93 and B94 were related to *Microbacterium luteolum* (Y17235) and *Arthrobacter* sp. BS11 (AY452080), respectively.

Pb library covered *Firmicutes* and *Proteobacteria*. Similar to the Cw library, *Firmicutes* were also numerically dominant, including 12 phylotypes, represented by 67 clones. However, besides *Paenibacillus*, two phylotypes (K7 and K21, represented by 11 clones) were related to *Bacillus*. Within *Gammaproteobacteria*, unlike the Cw library in which sequences were related to *Pantoea*, nine clones of Pb library were related to *Pseudomonas* and *Acinetobacter*.

Contrary to the above two culturable libraries, 99% of Cd clones were *Proteobacteria*. All *Gammaproteobacteria* were highly related to *Acinetobacter*, including five phylotypes, represented by 52 clones. Among six *Betaproteobacteria* phylotypes, five (represented by 21 clones) belonged to *Ralstonia* and only one single-sequence phylotype, B64, was related to *Comamonas testosteroni* (AF519533). In addition, phylotype B62 was related to *Chryseobacterium* (AY427792), a member of *Bacteroidetes*.

In total, no phylogenetic overlap at the division level was found between four libraries, with the exception of *Proteobacteria* (Table 2). Still, the difference in *Proteobacteria* was also detectable between four libraries when we checked them at the lower class level of taxonomy. *Alphaproteobacteria* and *Deltaproteobacteria* were exclusively in the Ci library, with the absence of *Gammaproteobacteria*, the dominant category in three culturable libraries. Moreover, the Pb library was dominated by *Pseudomonas*, but both Cw and Cd libraries were dominated by *Acinetobacter* (Fig. 1).

Phylotype Richness and Library Similarity. If all clones from each phylotype were viewed as the repeats of that phylotype's representative sequence, a total of 352 deduced sequences were obtained from four clone libraries. Subsequently, the phylotype richness of each library was estimated by rarefaction. As shown in Fig. 2, the Ci library had a relatively higher level of phylotype richness than three cultivable libraries. When only 50 sequences were sampled, there was a 95% chance that 18 to 27 phylotypes would be identified in the Ci library, which is already higher than that of the Cd, Pb, and Cw libraries. However, no difference in the richness was observed between three cultivable libraries when 70 sequences were sampled because the 95% confidence interval of the number of observed phylotypes from three libraries still overlapped.

The phylogenetic composition similarity between libraries was compared by using ∫-LIBSHUFF (Table 3). The difference is very significant between the four clone libraries ($P < 0.0001$). If we integrated Cd, Pb, and Cw as one library (cultivable library) to compare with the Ci library, the difference is still significant ($P < 0.0001$) (data not shown). These data indicate that phylogenetic structure was very different between microbes represented by cultivation-independent and -dependent methods. Moreover, in tailings, it is also very different between species communities resistant to Cd and Pb.

Discussion

In the present study, to avoid underestimating microbial diversity due to poor nutrient in bulk tailings and, moreover, to provide valuable suggestions to future phytoextraction of mine ecosystem, we collected rhizosphere tailings of naturally colonized plants to determine the bacterial diversity. Unexpectedly, the observed phylogenetic breadth was great and covered several previously described divisions

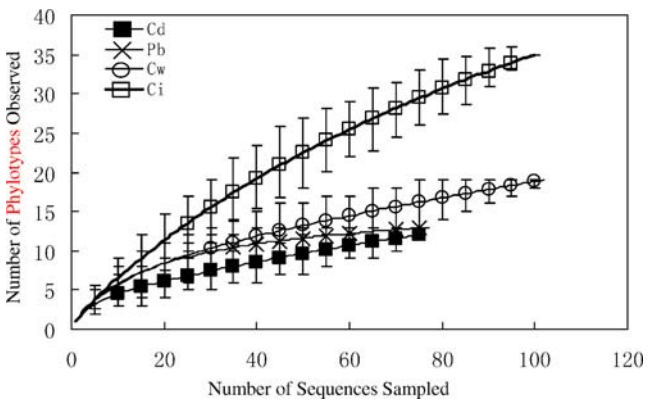

Figure 2. Rarefaction curves from DOTUR analysis by using the furthest neighbor assignment algorithm with the 16S rRNA gene libraries of cultivation-independent (*Ci*) and cultivation with 0.5mM $CdCl_2$ (*Cd*), 2mM $Pb(NO_3)_2$ (*Pb*), or without metals (*Cw*). *Error bars* represent the 95% confidence interval.

Table 3. Comparison of phylogenetic structure between four clone libraries by using ∫-LIBSHUFF

Libraries	*Cd*	*Pb*	*Cw*	*Ci*
Cd		<0.0001	<0.0001	<0.0001
Pb	<0.0001		<0.0001	<0.0001
Cw	<0.0001	<0.0001		<0.0001
Ci	<0.0001	<0.0001	<0.0001	

Comparisons were made using the integral form of the Cramér-von Mises statistic with 10,000 randomizations. The 95% margin of error for minimum *P* value is 0.0000.

either in heavy metal-contaminated [10, 21] or noncontaminated environments [11], such as *Acidobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*. *Acidobacteria* formed three subgroups and exclusively contained Ci clones (Fig. 1). Based on previous phylogenetic tree, *Acidobacteria* had eight subdivisions [16]. Our cluster 1 should be subdivision 4 of *Acidobacteria* because this subbranch included a representative (Z95720) from this subdivision. Although cluster 3 contained all seven remaining representative acidobacterial sequences, which belonged to seven subdivisions of *Acidobacteria*, our phylotype, B183, was closely related to *Acidobacterium capsulatum* (D26171), which represents subdivision 1. Cluster 2 contained four phylotypes but no previously described subdivision reference, and therefore, their detailed position remains to be determined.

Gemmatimonadetes were not found in previous heavy metal-contaminated soils. Currently, the presence of this division is mainly on the basis of 16S rRNA genes amplified directly from environmental DNA [2], and only four cultured representatives, named as genus *Gemmatimonas*, are obtained from activated sludge [35] and soils [30]. Therefore, we could not conclude that, in tailings, these organisms were unique and had important ecological functions. However, many previously described organisms in AMD, such as *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans* [20, 31], were not observed in our tailings. Low pH is the typical feature of AMD; thus, these common culturable organisms are acid-generating bacteria. Due to a slight basic pH in our tailings, such a result is not surprising. In addition, bacterial diversity is also different from those described in gold mine tailings in which numerically dominant clones seem to be *Proteobacteria* and *Bacteroidetes* [21]. Therefore, in different tailings, bacterial community is phylogenetically distinct. Nevertheless, the fact that eight reference sequences (sequences with black stars, Fig. 1) previously isolated from heavy metal-contaminated environments, including lead–zinc and uranium mine tailings, suggests that some bacteria may be common in some categories of tailings.

Our tailings contained Pb, Zn, and Cd; however, we only focused on Pb and Cd due to a relatively low concentration of Zn (also see Zhang et al. [36]) and great biotoxicity of Pb and Cd [8] when investigating subcommunities with different metal resistances. *Gammaproteobacteria* (mainly *Acinetobacter*) and *Betaproteobacteria* (mainly *Ralstonia*) were the dominant Cd-resistant bacteria (99% of 75 clones), but *Firmicutes* (mainly *Paenibacillus*) was dominant in Pb-resistant bacteria (87% of 77 clones). Metal resistance of *Gammaproteobacteria* may be explained by the prevalence of a multiple metal- and drug-resistance element, integron, in these species [21]. Many strains of *Acinetobacter* isolated from different environmental sources were reported previously with resistance to copper, lead, boron, and tungsten [6]. Members of *Ralstonia*, formerly as *Alcaligenes*, were also proved to contain efflux pump for Co^{2+}, Zn^{2+}, and Cd^{2+} resistance [22]. These organisms were also found to be dominant in Hg-contaminated soil [26]. However, we do not know if our *Acinetobacter* or *Ralstonia* also contain similar metal efflux pump, and thus, the reason of their dominance in Cd library is uncertain. Pb-resistant bacteria contained *Paenibacillus*, *Pseudomonas* and *Bacillus*. Previously high frequency of *Bacillus* was noted in sediments containing high concentrations of Hg and other heavy metals [32] and in gold mine tailings [10]. Meanwhile, *Pseudomonas* is also common in metal-contaminated environments. The genome analysis of *Pseudomonas putida* KT2440 categorized 61 open reading frames likely to be involved in metal tolerance or homeostasis [4]. However, no heavy-metal resistance of *Paenibacillus* was described previously.

It is interesting to note that, although Cd-resistance gene *cad*A and its homologs are prevalent in Gram-positive bacteria [24], all Cd clones were exclusively dominant in Gram-negative bacteria, including *Acinetobacter*, *Ralstonia*, *Comamonas*, and *Chryseobacterium*, but dominant Pb-resistant bacteria were Gram-positive, such as *Paenibacillus* and *Bacillus*. These data indicated a very different competitive advantage between Gram-negative and -positive bacteria under Cd or Pb stress.

Therefore, the heterogeneously distributed metals in tailing heap should select phylogenetically distinct subcommunities resistant to different metal pools. For example, we could expect that *Paenibacillus* and *Bacillus* should mainly distribute in Pb pool, but members of *Proteobacteria*, such as *Acinetobacter* and *Ralstonia*, tended to survive in Cd pool. Certainly, besides these organisms, many uncultured bacteria should also distribute like this style, but we cannot prove it by current methods.

Firmicutes and *Proteobacteria* are the predominant cultivable bacteria, a typical characteristic of the cultivation-based methods [9, 10, 17]. It is surprising to note that no phylogenetical overlap was found between Cw and two metal-resistant libraries (Table 2, Fig. 1). Although the Cw library was also dominated by *Paenibacillus*, its sequence similarity was significantly different from that of the Pb library. There were 26/77 sequences of the Pb library phylogenetically close

to *Paenibacillus ginsengagri* (similarity ranging from 98.5 to 98.7%, accession no. AB245383) and 20/77 to *Paenibacillus lautus* (similarity ranging from 93.9 to 100%, accession no. AB073188). Instead, most sequences (64/102) from the Cw library were phylogenetically close to *Paenibacillus* (similarity ranging from 91.9 to 98%, accession no. AM162303). The possible reason was that those species or strains grown on medium with Pb or Cd may lose growth advantage when without metals. This should result from adaptive cost of resistance to metals. Furthermore, although many efflux pumps could provide host cells with multiple-metal resistance for Co^{2+}, Zn^{2+}, and Cd^{2+} [22], no overlap between Cd and Pb libraries indicates that no such strains or species were resistant to both metals, or at least to 0.5 mmol l^{-1} of Cd and 2 mmol l^{-1} of Pb.

In a previous report, we described that *Arthrobacter* was a dominant cultivable species (on average 61% of cultivable colonies) in tailings [36]. In this study, however, only 1 of 102 clones from the Cw library was *Arthrobacter* (Fig. 1). Such an inconsistency may mainly have resulted from sampling. Previous tailings were collected from interspaces of plants. Therefore, this inconsistency suggested that it was significantly different between bacterial communities of rhizosphere and bulk tailings, at least to cultivable portions. *Paenibacillus* is common in rhizospheres of plants; many species promote plant growth through nitrogen fixing [33]. Therefore, the dominance of *Paenibacillus* in rhizosphere tailings suggested its potentials in enhancing plant survival in mine tailings.

Only 33 unique sequences were identified from 98 Ci clones and 42 from 254 cultivable clones (Table 1); such a large proportion of sequence repeats may be explained by the limited resolution of restriction enzyme. In some cases, a phylotype probably does not represent a single species and bacterial diversity of mine tailings should be underestimated. The relatively short 16S rRNA gene fragment (about 400 bp) used in RFLP analysis also aggravates this status. In addition, because both plating and direct 16S rDNA cloning suffer from biases that can distort community composition, richness, and structure, caution must be taken in explaining these parameters. For example, the dominance of *Paenibacillus* in the Cw and Pb library or *Acinetobacter* and *Ralstonia* in library Cd may be selected by heterotrophic bias of the medium. Therefore, other organisms may be missed even if they can also resist Pb or Cd. In addition, due to chelation of metals by medium components, some species or strains we observed actually cannot tolerate such concentrations of metal. On the other hand, because only one concentration of each metal was detected, some bacterial species or strains with the resistant potential for a unique tolerance level towards each metal should be missed. In the future, combination of fluorescence *in situ* hybridization and x-ray absorption fine structure [3] could provide more detailed information to enhance our understanding of geochemistry of tailings and improve bioremediation *in situ*.

Acknowledgements

We thank Zhi-Ying Zhu, Chan-Wen Xu, Yan Li, Shu-Hua Ge, Tao Li, and Chang-Chong Liang in Yunnan University for sampling, library constructions, and RFLP analyses. This study was funded by the Natural Science Foundation of China (30560033) and the National Program on Key Basic Research Projects of China (special item 2005CCA05700).

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