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ORIGINAL PAPER

Culturable and molecular phylogenetic diversity of microorganisms in an open-dumped, extremely acidic Pb/Zn mine tailings

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Abstract A combination of cultivation-based and molecular-based approaches was used to reveal the culturable and molecular diversity of the microbes inhabiting an open-dumped Pb/Zn mine tailings that was undergoing intensive acid generation (pH 1.9). Culturable bacteria found in the extremely acidic mine tailings were *Acidithiobacillus ferrooxidans*, *Leptospirillum ferriphilum*, *Sulfobacillus thermotolerans* and *Acidiphilium cryptum*, where the number of acidophilic heterotrophs was ten times higher than that of the iron- and sulfur-oxidizing bacteria. Cloning and phylogenetic analysis revealed that, in contrast to the adjacent AMD, the mine tailings possessed a low microbial diversity with archaeal sequence types dominating the 16S rRNA gene library. Of the 141 clones examined, 132 were represented by two sequence types phylogenetically affiliated with the iron-oxidizing archaea *Ferroplasma acidiphilum* and three belonged to two tentative groups within the *Thermoplasma* lineage so far represented by only a few environmental sequences. Six clones in the library were represented by the only bacterial sequence type and were closely related to the well-described iron-oxidizer *L. ferriphilum*. The significant differences in the prokaryotic community structures of the extremely acidic mine tailings and the AMD associated with it highlights the importance of studying the microbial communities that are more directly involved in the iron and sulfur cycles of mine tailings.

Keywords Mine tailings · Acid mine drainage · Acidophiles · Microbial diversity · 16S rRNA gene

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Introduction

Acid mine drainage (AMD) generated by microbially mediated oxidation of pyrite (FeS_2) and other sulfide minerals has been the cause of serious environmental problems worldwide. As a result, the past few decades have seen the study of composition and diversity of acidophilic microorganisms populating various AMD environments receiving much attention (Baker and Banfield 2003). Collectively, these studies have provided useful insights into the biological functioning of these unique ecosystems and how microbial communities are shaped by the "harsh" geochemical factors therein.

Mine tailings resulting from mining and metallurgical processes represent an important source of AMD production. However, unlike in AMD itself, little information is currently available on the microbial diversity in acidic mine tailings. Most of the conducted work has been focused on the enumeration and distribution of acidophilic chemolithoautotrophic Gram-negative bacteria via cultivation-dependent methods (Silver 1987; Southam and Beveridge 1992; Schippers et al. 1995; Fortin et al. 1996; Wielinga et al. 1999). Although cultivation-based

approaches are extremely useful in revealing the physiological traits of the microorganisms isolated from the tailings and their potential functions in the production of AMD in situ, there is a need to validate these findings using the now well-established culture-independent molecular techniques. Several recent studies have applied molecular approaches to investigate the structure and abundance of acidophiles in mine tailings (Bryan et al. 2006; De la Iglesia et al. 2006; Kock and Schippers 2006); however, only one report detailed clone library data to reveal the microbial composition and diversity in acidic mine tailings (Diaby et al. 2007).

The Lechang Pb/Zn mine is located about 4 km east of Lechang City in the northern Guangdong Province, People's Republic of China. It has been in operation for more than 50 years. Mine tailings produced from the milling process are discharged (as a slurry) into several adjacent tailings ponds where they undergo intensive acid generation after contact with air and water. Approximately 30,000 tons of tailings are generated annually with a dumping area of 60,000 m^2. To minimize the oxidation of pyrite and the production of acidic effluents, some remediation measures have been taken in the past few years (Yang et al. 2003); but these attempts turned out to be unsuccessful. We have previously characterized the microbial diversity populating the AMD associated with these open-dumped mine tailings and found that the AMD ecosystem, although low in total taxonomically distinct groups, harbored a wide range of phylogenetically diverse microbes (Tan et al. 2007). In particular, sequences from the newly discovered iron-oxidizing *Leptospirillum* group III within the *Nitrospira* dominated the 16S rRNA gene clone library. However, the phylogenetic diversity of the microorganisms within the acidic mine tailings, which are more likely directly involved in the acid generation process in situ, remains unknown thus far. Here we report the composition and diversity of the indigenous microbial community in the AMD-generating mine tailings by applying cultivation-dependent and cultivation-independent approaches. Surprisingly, significantly differing from the adjacent AMD, the acidic mine tailings harbored a simple community with archaeal sequence types dominating the 16S rRNA gene library.

Materials and methods

Sample collection and geochemical analysis

Three different regions of the Lechang mine tailings showing strong signs of oxidization or acidification (reddish brown color) were selected and composite tailings samples were taken from each region at a depth of 0–20 cm on 15 October 2005. On-site air and tailings-associated AMD temperature was 27–29°C, and the tailings moisture content was 20.6%. These environmental parameters were comparable to those of 18 September 2004 when the tailings-associated AMD was sampled for the previous study (Tan et al. 2007). Once collected, samples were immediately transported to the laboratory and stored at 4°C and processed within 24 h for microbial cultivation and DNA extraction (the cell recovery step). The three composite tailings samples were mixed individually and analyzed separately throughout this study, except that purified total community DNAs from the tailings samples were pooled before PCR amplification for clone library analysis (see below). The pH of the tailings samples was measured after shaking 5 g fresh sample in 12.5 ml 1 M KCl for 1 h. Soluble sulfate concentrations in the water extracts of tailings (at a solid-water ratio of 1:5, wt/vol) were determined by a $BaSO_4$-based turbidimetric method. Total metal concentrations were determined by atomic absorption spectrophotometry (AAS) after the tailings were digested at 180°C with conc. HNO_3, HCl and $HClO_4$ (4:1:1, v/v).

Microbial isolation and pyrite oxidation test

Acidophilic iron-, sulfur-oxidizing bacteria and heterotrophs were enumerated and isolated using the overlay media (iron, Fe_o, iron/tetrathionate, FeS_o and yeast extract, YE_o) as previously described (Hallberg and Johnson 2003). Overlay media have been shown to be both efficient and selective for cultivating both autotrophic and heterotrophic acidophiles. Briefly, the three tailings samples were suspended individually in sterile basal salts solution (pH 3.0). The suspensions were incubated (28°C) for 1 h on a rotary shaker at 180 rpm, then serially diluted (tenfold) in the above-mentioned sterile solution and plated (in triplicate) on solid media, followed by a 10–20 day incubation in the dark at 28°C (comparable to the temperatures recorded on site when sampling) prior to enumeration. The plate counts are presented as mean colony forming units (CFU) per gram (dry weight) of tailings of the three different samples. Representative colonies from each of overlay plates were then purified by streaking at least two times before obtaining final pure isolates. Colonies that grew on different overlay plates were viewed using a stereo-scan microscope (Leica ZOOM 2000). Molecular identification of the isolates was performed as described previously (Tan et al. 2007).

Three tailings isolates were chosen and assessed for their ability to oxidize pyrite. Pure cultures were inoculated into a liquid medium (100 in 250-ml shake flasks, pH 2.5) containing 1% (w/v) sterilized ground rock pyrite, and the inoculated flasks were incubated at 28°C for 30 days with shaking (180 rpm). Samples were removed at regular

intervals to measure pH and concentrations of soluble ferric iron were determined using the *ortho*-phenanthroline colorimetric method. The shake flask cultures were corrected (with sterile distilled water) per sampling event for liquid loss due to evaporation. Uninoculated shake flasks were used as control.

DNA extraction and clone library analysis

Prior to the real sampling, attempts using different approaches (e.g., the rapid method reported by Tsai and Olson (1991) and the MoBio UltraClean Soil DNA Kit) to extract total community DNA directly from the Lechang acidized tailings failed due to co-precipitation of high amounts of salts presented in the tailings (data not shown). Subsequently, an indirect method employing cell recovery with dispersing reagent prior to DNA extraction was used in this study. Briefly, cells were recovered from 16 g tailings by using sodium pyrophosphate (pH 3.0) as dispersal reagent (Duarte et al. 1998). This recovery step was repeated twice. The cell pellets obtained were lysed by using lysozyme and a freeze-thaw procedure, and the lysate was extracted with sodium dodecyl sulfate and phenol-chloroform (Tsai and Olson 1991). The extracted DNA was subsequently purified on spin columns containing Sepharose 4B (Sigma). Purified nucleic acids from the three individual tailings samples were pooled, and 16S rRNA gene fragments were amplified from the bulk DNA sample using the universal primers 533F (5′ GTG CCA GCM GCC GCG GTA A 3′) and 1492r (5′ GGT TAC CTT GTT ACG ACT T 3′) (Dojka et al. 1998) and the cycling parameters described previously (Tan et al. 2007). To minimize PCR bias, triplicate PCR products were pooled prior to cloning. Clone library construction, recombinant clones screening, DNA sequencing, and chimeric sequence identification were carried out exactly as described in the study of the tailings-associated AMD (Tan et al. 2007). The 16S rRNA gene sequences were compared with those in the GenBank using the BLASTn program (Altschul et al. 1990). Sequences differing only slightly (≤2%) were considered as an operational taxonomic unit (OTU), and each OTU was represented by a type sequence. Neighbor-joining phylogenetic trees were constructed as described previously (Tan et al. 2007). The relative confidence in nodes for neighbor-joining and maximum parsimony analysis was evaluated by performing 1,000 bootstrap replicates. Library coverage (Good 1953) was calculated using $C = 1\text{-}n/N$, where n is the number of sequence types that occur only once in the library and N is the total number of clones examined. LIBSHUFF software (Singleton et al. 2001) was used to determine the significance of differences between the mine tailings clone library and the tailings-associated AMD clone library constructed in our previous study (Tan et al. 2007). To compare the rRNA-based richness within the two libraries, rarefaction analysis was performed by using DOTUR (Schloss and Handelsman 2005).

Nucleotide sequence accession numbers

The partial 16S rRNA gene sequences of the isolates and clones from this study have been deposited in the EMBL database under accession numbers AM502927 to AM502933 and AM503914 to AM503919.

Results and discussion

Geochemistry of tailings samples

The mine tailings sampled showed visible signs of oxidization (reddish brown color), had an average pH value of 1.9 and high levels of total Fe (mean 87,000 mg kg^{-1} dry weight of tailings) and sulfate (mean 67,000 mg kg^{-1}). The mean concentrations of Pb and Zn were 3,109 and 1,548 mg kg^{-1}, respectively. Other metal concentrations, such as Mn, Cu, Cd, Cr and Ni, were 223, 50, 2.75, 4.91 and 1.29 mg kg^{-1}, respectively.

Isolation and characterization of acidophilic bacteria

Iron- and sulfur-oxidizing bacteria (grew on Fe_o and FeS_o plates, respectively, pH 2.5) were found to be present in the top 20 cm of tailings in similar numbers (mean counts of the three samples were $2.2 \pm 0.3 \times 10^3$ and $2.2 \pm 0.2 \times 10^3$ CFU g^{-1} tailings, respectively). The same orders of magnitude in numbers of iron- and sulfur-oxidizing microbes were previously detected in other mine tailings (Wielinga et al. 1999). In contrast, the concentrations of culturable acidophilic heterotrophs (grew on YE_o, pH 3.0) were ten times higher than that of the culturable autotrophic bacteria, reaching $1.6 \pm 0.1 \times 10^4$ CFU g^{-1} of tailings. While the higher cell numbers of heterotrophic microbes might have been due in part to the fact that they often grow more efficiently on plates, recently acidophilic heterotrophs have also been found to predominate over chemolithotrophs in the oxidation zone of an acidic (pH 2–4) porphyry copper tailings in Chile (Diaby et al. 2007). After purifying by repeated streaking onto the respective solid media, a total of seven isolates showing different colony morphologies were obtained (Table 1) and their 16S rRNA genes were cloned and sequenced. Isolates LMT1 and LMT4 (grew on Fe_o and FeS_o plates, respectively) exhibited 98.3 and 99.1% sequence similarity with the type strain *A. ferrooxidans* ATCC 23270, respectively. Isolates LMT2, LMT3 and LMT5 (obtained with Fe_o or

FeS_o plates and shared 97.5–99.4% sequence similarity with each other) were highly related (98.0–99.5% sequence identify) to the type strain of *L. ferriphilum*. In addition, isolate LMT6 (obtained with FeS_o plate) was phylogenetically associated with the iron- and sulfur-oxidizing bacteria *Sulfobacillus thermotolerans* KR-1 (98.7% sequence similarity), and isolate YT1 (from YE_o plate) was closely related (99.8% sequence similarity) to *Acidiphilium cryptum*, a heterotrophic acidophile capable of reducing ferric to ferrous while oxidizing reduced carbon compounds (Johnson and Bridge 2002). While growth of the heterotrophic isolate YT1 was observed even in the 10^{-3}-dilution YE_o plates, growth of the autotrophic isolates (LMT1–6) were only obtained in the 10^{-1}-dilution plates except for LMT2 and 6 which formed distinguishable colonies in the 10^{-2}-dilution Fe_o and FeS_o plates, respectively.

Pyrite oxidation test of the *Acidithiobacillus* isolates LMT1 and LMT4 and the *Sulfobacillus* isolate LMT6 revealed that they were all capable of solubilizing iron from pyrite (Fig. 1). The most efficient pyrite-oxidizing isolates were LMT1 and LMT4, showing identical leaching trends, whereas strain LMT6 did not oxidize pyrite until after 15-day incubation. The pH value in all pyrite cultures decreased from 2.5 to 1.5–1.7 after 30 days.

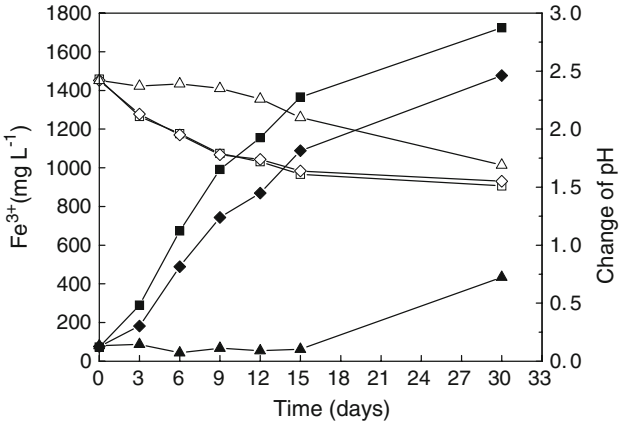

Fig. 1 Oxidation of pyrite, measured as decrease in pH (*open symbols*) and release of ferric iron (*solid symbols*), by Lechang tailings isolates LMT1 (*squares*), LMT4 (*diamonds*) and LMT6 (*triangles*)

Phylogenetic diversity of the tailings community

Restriction endonuclease analysis of the 142 randomly selected clones from the 16S rRNA gene library revealed a total of 15 unique RFLP types. DNA sequencing of the representative clones and sequence similarity then reduced the RFLP groups to only seven sequence types. One of these sequence types, represented by a single clone, was found to be strongly chimeric and was excluded from further analyses. Library coverage was calculated to be >97%, indicating that the clone library was sufficiently sampled. These results indicated that the extremely acidic Lechang tailings harbored a simple microbial community.

Comparative analysis of the retrieved sequences revealed that clones affiliated with the *Archaea*, in particular those represented by the sequence types LCT9 and 44,

Table 1 Colony characteristics and nearest neighbors of the representative Lechang tailings isolates

Isolate	Colony morphology	Nearest neighbors and nearest known microorganisms[a]	Similarity (%)	Isolation medium
LMT1	Large, entire, rusty orange-brown	*Acidithiobacillus ferrooxidans* D2	99.1	Fe_O
		A. ferrooxidans ATCC 23270^T	98.3	
LMT2	Small, entire, rusty orange-brown	*Leptospirillum ferriphilum* Fairview	99.6	Fe_O
		L. ferriphilum ATCC 49881^T	99.5	
LMT3	Medium, entire, rusty orange-brown	Tinto River AMD clone fe7	99.5	Fe_O
		L. ferriphilum Warwick	99.5	
		L. ferriphilum ATCC 49881^T	98.3	
LMT4	Large, entire, rusty orange-brown, bleached or yellow with time	Tinto River AMD clone fppg9	99.7	FeS_O
		A. ferrooxidans DSM9465	99.4	
		A. ferrooxidans ATCC 23270^T	99.1	
LMT5	Small, entire, orange-brown	Tinto River AMD clone fe7	99.2	FeS_O
		L. ferriphilum Warwick	99.2	
		L. ferriphilum ATCC 49881^T	98.0	
LMT6	Small, entire, orange-brown, white in periphery	*Sulfobacillus thermotolerans* KR-1^T	98.7	FeS_O
YT1	Small, entire, milk-white	*Acidiphilum cryptum* ATCC 33463^T	99.8	YE_O

[a] Closest relatives as determined by the BLAST method (Altschul et al. 1990)

dominated the 16S rRNA gene library (Fig. 2). LCT9 (the most abundant sequence type in the gene library; 128 clones) and LCT44 (4 clones) were phylogenetically affiliated with the genus *Ferroplasma*. They were closely related (96.5–99.7% sequence similarity) to *F. acidiphilum*, an ferrous-iron-oxidizing archaea first isolated from a bioleaching pilot plant (Golyshina et al. 2000), and to environmental AMD clones recently retrieved from the Tinto River (García-Moyano et al. 2007) and the Richmond Mine at Iron Mountain, California (Bond et al. 2000). Archaeal members have previously been found to dominate the microbial communities populating AMD habitats or bioleaching systems (Edwards et al. 1999; Hawkes et al. 2005). Although the proportions of clone sequences in the library cannot be translated directly into the true frequency of the corresponding species, the dominance of the two Lechang archaeal sequence types likely reflects the high abundance of *F. acidiphilum*-like archaea in the original tailings samples and these indigenous iron-oxidizers might largely contribute to the oxidation of pyrite and the production of AMD within these tailings. Since *Ferroplasma* spp. are more acidophilic than other iron- and sulfur-oxidizing bacteria exhibiting similar eco-physiological properties, they are considered to be important players in the biogeochemical cycling of sulfur and sulfide minerals in highly extreme mining environments characterized by very low pH, high concentrations of ferrous and total iron and other heavy metals, and moderately elevated temperatures (Golyshina and Timmis 2005). Unfortunately, we failed to isolate any of these *Ferroplasma* species on the selective solid media. A likely explanation is that the overlay plates we used did not support the growth of these organisms, probably due to the lack of yeast extract in the media Fe_o and FeS_o which support the growth of iron- and sulfur-oxidizing bacteria, respectively. Previously published data have shown that supplementing medium with yeast extract helps in promoting the growth of *Ferroplasma* species (Golyshina et al. 2000; Dopson et al. 2004; Hawkes et al. 2006). It is also possible that the *Ferroplasma* organisms did not survive the resuspending step with the

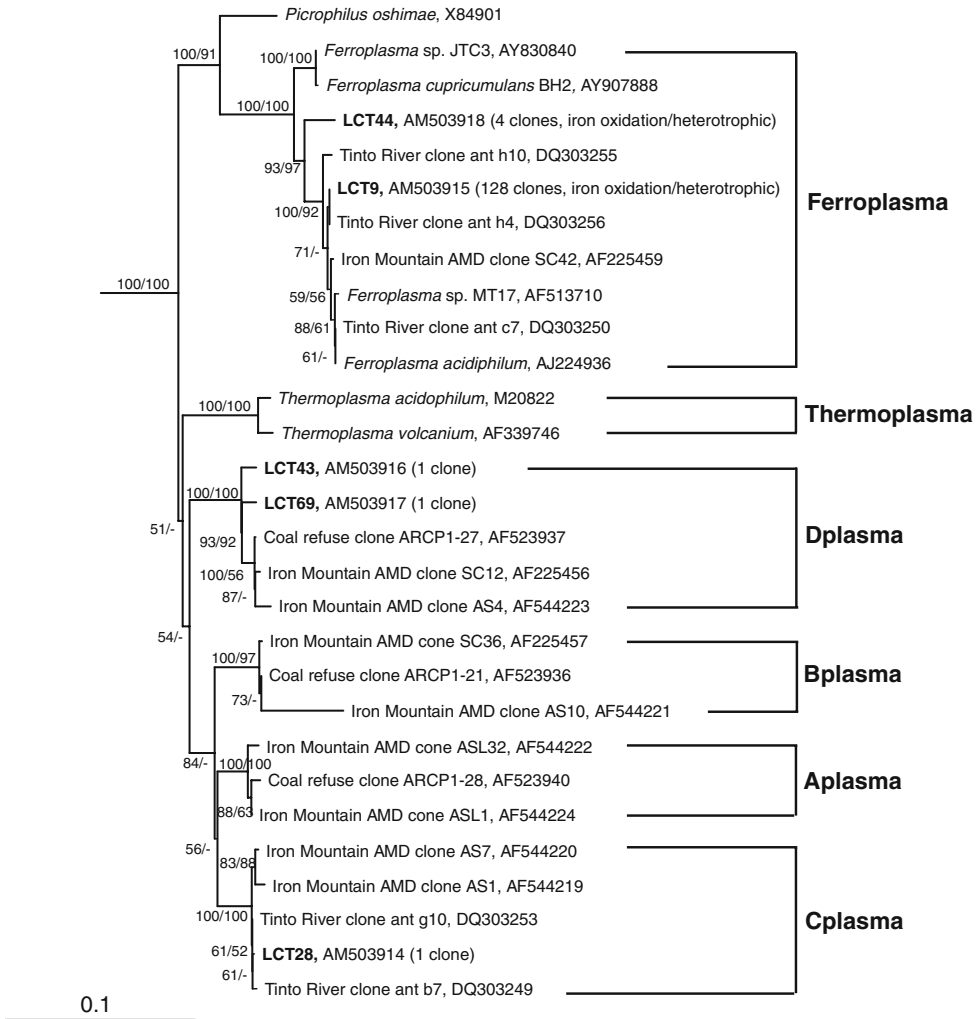

Fig. 2 Phylogenetic relationship of archaeal 16S rRNA gene sequences retrieved from the Lechang Pb/Zn mine tailings. Putative phylogenetic groups are listed to the right. Bootstrap values (neighbor-joining and maximum parsimony) of ≥50% are reported as percentages at the respective node. The number of closely related (≥98% similarity) clones found in the library, together with the inferred metabolisms of their corresponding organisms, is indicated at the end of the sequence types in *parentheses*. The *scale bar* represents the number of changes per nucleotide position

pH 3.0 basal salts solution prior to plating since cells of extreme archaea have been demonstrated to lyse and lose their viability at moderately high pH values (van de Vossenberg et al. 1998). Further research is needed to investigate these points.

Each of the other three *Archaea*-affiliated sequence types was represented by a single clone. LCT28 was well positioned within the Cplasma group (Baker and Banfield 2003), sharing sequence identities of 99.6% with the Tinto River clone ant b7 (García-Moyano et al. 2007) and 99.3% with the AMD clone AS7 retrieved from the Richmond Mine (Baker and Banfield 2003). In contrast, both LCT43 and LCT69 were clustered within the Dplasma group (Baker and Banfield 2003), showing 98.1–98.2% similarity with the environmental clone ARCP1-27 detected in a reject coal pile-associated AMD (Brofft et al. 2002).

LCT61 represented the second most abundant sequence type in the gene library (6 clones; 4.2% of the library) and was the only sequence type grouped within the domain *Bacteria* (Fig. 3). It was phylogenetically affiliated with the *Nitrospira* group, specifically *Leptospirillum* group II, and showed 99.6% similarity with 16S rRNA gene of the iron-oxidizing bacteria *L. ferriphilum* Warwick (Coram and Rawlings 2002) and >98.8% similarity with those of the *L. ferriphilum*-like tailings isolates obtained on overlay plates (LMT2, LMT3 and LMT5). Given that *L. ferriphilum* has higher affinity to ferrous iron and more tolerance to ferric iron and low pH than *At. ferrooxidans* (Rawlings et al. 1999; Coram and Rawlings 2002), this iron-oxidizer might contribute to the oxidation of pyrite within the Lechang tailings characterized by high level of iron and extremely low pH.

Comparing the tailings and AMD populations

All of the 16S rRNA gene sequences from the tailings isolates, together with the only bacterial sequence type identified in the clone library, clustered with those previously recovered from the tailings-associated AMD at this site (Tan et al. 2007) (Fig. 3), indicating that the two acidophilic communities are related. However, there is significant difference in constituents between the tailings and the AMD communities (Fig. 4). While *Archaea* dominated the tailings clone library, the domain *Bacteria* exclusively made up the AMD library. Statistical analysis using LIBSHUFF also confirmed that most sequences from the two libraries had low similarity [the heterologous coverage was significantly different ($P = 0.001$) from the

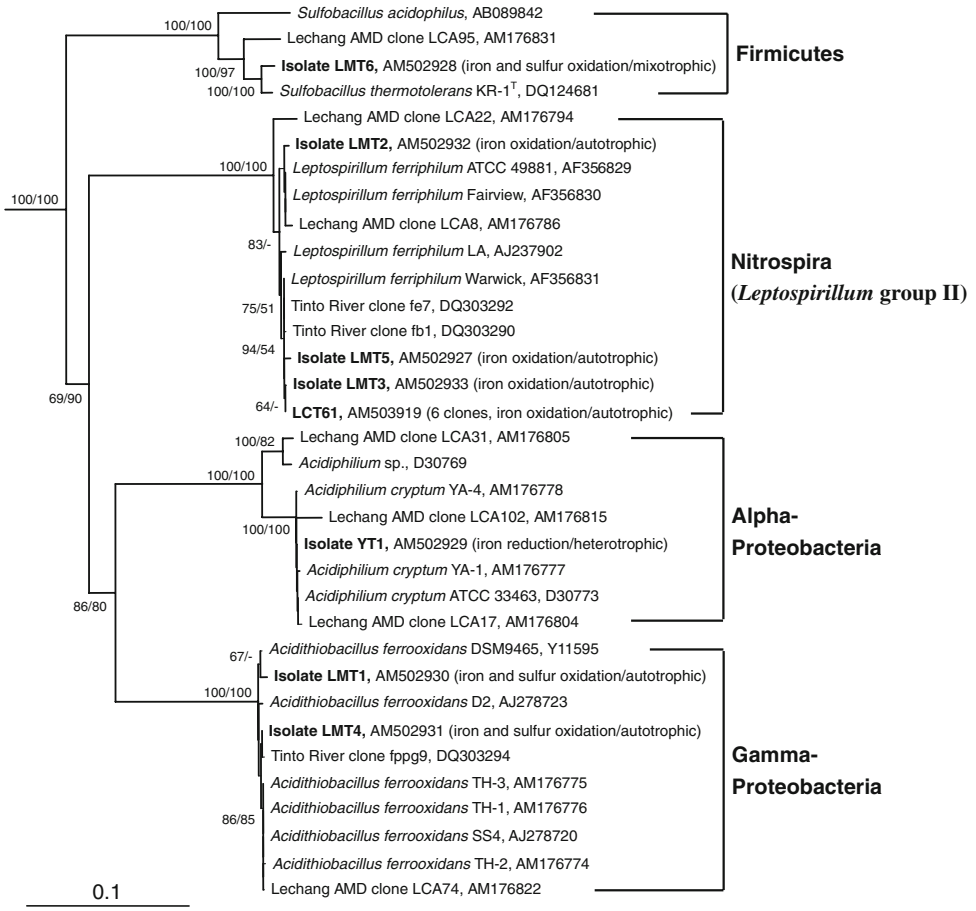

Fig. 3 Phylogenetic relationship of bacterial 16S rRNA gene sequences retrieved from the Lechang Pb/Zn mine tailings. Putative divisions or subdivisions are listed to the *right*. Bootstrap values (neighbor-joining and maximum parsimony) of ≥50% are reported as percentages at the respective node. The number of closely related (≥98% similarity) clones found in the library, together with the inferred/confirmed metabolisms of their corresponding organisms, is indicated at the end of the sequence types in *parentheses*. The *scale bar* represents the number of changes per nucleotide position

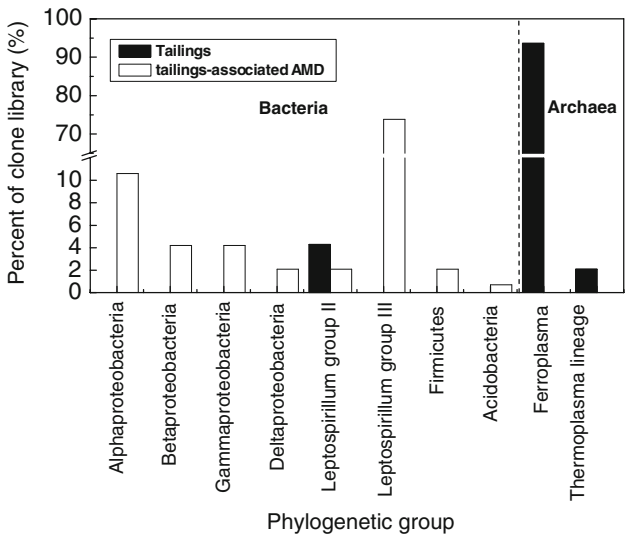

Fig. 4 Pylogenetic distribution of 16S rRNA gene clones in the Lechang tailings library compared to that in the tailings-associated AMD library (Tan et al. 2007). Grouping analysis revealed that the clone distribution between these two libraries differs significantly

homologous coverage]. Rarefaction analysis revealed that the tailings library has a significantly lower relative species richness than the AMD library (data not shown). More importantly, bacteria from *Leptospirillum* group III, the novel iron-oxidizing chemolithoautotrophic group that we previously speculated (based on their dominance in the AMD clone library) to largely contribute to the production of AMD at this site, were not detected in the present study. These new findings suggest that members of this novel *Leptospirillum* group are not the major acidophiles mediating the pyrite dissolution within the Pb/Zn mine tailings and may be only relevant to the iron-oxidation peripheral the dissolution process.

It has been widely accepted that each of the multiple steps involved in the clone library approach may introduce biases. We followed the same procedures/conditions in constructing and analyzing the two clone libraries. The only exception is that cells were recovered from the tailings samples prior to DNA extraction, since all direct extraction methods that we tried failed to obtain enough amounts of DNA necessary for PCR amplification. However, in a recent study comparing the efficiency of DNA recovery by direct and indirect methods from environmental samples with largely differing characteristics, Gabor et al. (2003) found that although cell extraction-based isolation methods yielded lower amounts of DNA, the bacterial diversity recovered was distinctly higher than those obtained by direct lysis procedures. As such, the observed differences in the tailings and AMD microbial communities are likely related to the physicochemical conditions existing in these two adjacent habitats. The difference in sampling time (15 October 2005 vs. 18 September 2004) of the two studies should be taken into account, although major environmental conditions (temperature and tailings moisture content) recorded are comparable. Nevertheless, it is somewhat surprising to observe a much simpler microbial community in the acidic mine tailings since, compared to AMD environments, mine tailings are considered to have more heterogeneity, and changes such as temperature, humidity and availability of oxygen in the tailings may shape indigenous microbial ecology (Fortin et al. 2000; Schippers et al. 1995; Silver 1987). One possible reason could be that since the tailings samples analyzed in the current study are limited in number and restricted to one sampling date and to the considerably oxidized zones only, it is unlikely that the microbial diversity reported are representative of the whole tailings environment at this site. Further investigations should be oriented towards exploring the microbial populations inhabiting tailings at different oxidation stages and how the dominant species fluctuate in response to the varying environmental conditions at these important sites of AMD production.

In conclusion, the extremely acidic Lechang Pb/Zn mine tailings harbored a low microbial diversity. *F. acidiphilum*- and *L. ferriphilum*-like iron-oxidizing acidophiles (in particular the *F. acidiphilum*-like archaea) likely play an important role in the pyrite oxidation and AMD generation within the mine tailings. This community contrasts greatly from that detected in the adjacent AMD, highlighting the importance of studying the microbial communities that are more directly involved in the iron and sulfur cycles of mine tailings as opposed to inferring this from a study of the associated AMD community.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

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

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 Microbiol 66:3842–3849

Brofft JE, McArthur JV, Shimkets LJ (2002) Recovery of novel bacterial diversity from a forested wetland impacted by reject coal. Environ Microbiol 4:764–769

Bryan CG, Hallberg KB, Johnson DB (2006) Mobilisation of metals in mineral tailings at the abandoned Sao Domingos copper mine (Portugal) by indigenous acidophilic bacteria. Hydrometallurgy 83:184–194

Coram NJ, Rawlings DE (2002) Molecular relationship between two groups of the genus *Leptospirillum* and the finding that *Leptospirillum ferriphilum* sp. nov. dominates South African

commercial biooxidation tanks that operate at 40°C. Appl Environ Microbiol 68:838–845

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 HR, 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

Dojka MA, Hugenholtz P, Haack SK, Pace NR (1998) Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. Appl Environ Microbiol 64:3869–3877

Dopson M, Baker-Austin C, Hind A, Bowman JP, Bond PL (2004) Characterization of *Ferroplasma* isolates and *Ferroplasma acidarmanus* sp. nov., extreme acidophiles from acid mine drainage and industrial bioleaching environments. Appl Environ Microbiol 70:2079–2088

Duarte GF, Rosado AS, Seldin L, Keijzer-Wolters AC, van Elsas JD (1998) Extraction of ribosomal RNA and genomic DNA from soil for studying the diversity of the indigenous bacterial community. J Microbiol Methods 32:21–29

Edwards KJ, Gihring TM, Banfield JF (1999) Seasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment. Appl Environ Microbiol 65:3627–3632

Fortin D, Davis B, Beveridge TJ (1996) Role of *Thiobacillus* and sulfate-reducing bacteria in iron biocycling in oxic and acidic mine tailings. FEMS Microbiol Ecol 21:11–24

Fortin D, Roy M, Rioux J, Thibault P (2000) Occurrence of sulfate-reducing bacteria under a wide range of physico-chemical conditions in Au and Cu-Zn mine tailings. FEMS Microbiol Ecol 33:197–208

Gabor EM, de Vries EJ, Janssen DB (2003) Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. FEMS Microbiol Ecol 44:153–163

García-Moyano A, González-Toril E, Aguilera A, Amils R (2007) Prokaryotic community composition and ecology of floating macroscopic filaments from an extreme acidic environment, Río Tinto (SW, Spain). Syst Appl Microbiol 30:601–614

Golyshina OV, Pivovarova TA, Karavaiko GI, Kondrateva TF, Moore ER, Abraham WR, Lunsdorf H, Timmis KN, Yakimov MM, Golyshin PN (2000) *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the *Archaea*. Int J Syst Evol Microbiol 50:997–1006

Golyshina OV, Timmis KN (2005) *Ferroplasma* and relatives, recently discovered cell wall-lacking archaea making a living in extremely acid, heavy metal-rich environments. Environ Microbiol 7:1277–1288

Good IJ (1953) The population frequencies of species and estimation of population parameters. Biometrika 40:237–264

Hallberg KB, Johnson DB (2003) Novel acidophiles isolated from moderately acidic mine drainage waters. Hydrometallurgy 71:139–148

Hawkes RB, Franzmann PD, Plumb JJ (2005) Moderate thermophiles including *Ferroplasma cyprexacervatum* sp. nov. dominate an industrial-scale chalcocite heap bioleaching operation. In: Harrison STL, Rawlings DE, Petersen J (eds) Proceedings of the sixteenth international biohydrometallurgy symposium, pp 657–666

Hawkes RB, Franzmann PD, O'hara G, Plumb JJ (2006) *Ferroplasma cupricumulans* sp. nov., a novel moderately thermophilic, acidophilic archaeon isolated from an industrial-scale chalcocite bioleach heap. Extremophiles 10:525–530

Johnson DB, Bridge TAM (2002) Reduction of ferric iron by acidophilic heterotrophic bacteria: evidence for constitutive and inducible enzyme systems in *Acidiphilium* spp. J Appl Microbiol 92:315–321

Kock D, Schippers A (2006) Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage. Hydrometallurgy 83:167–175

Rawlings DE, Tributsch H, Hansford GS (1999) Reasons why '*Leptospirillum*'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. Microbiology 145:5–13

Schippers A, Hallmann R, Wentzien S, Sand W (1995) Microbial diversity in uranium mine waste heaps. Appl Environ Microbiol 61:2930–2935

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71:1501–1506

Silver M (1987) Distribution of iron-oxidizing bacteria in the Nordic uranium tailings deposit, Elliot Lake, Ontario, Canada. Appl Environ Microbiol 53:846–852

Singleton DR, Furlong MA, Rathbun SL, Whitman WB (2001) Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. Appl Environ Microbiol 67:4374–4376

Southam G, Beveridge TJ (1992) Enumeration of *Thiobacilli* within pH-neutral and acidic mine tailings and their role in the development of secondary mineral soil. Appl Environ Microbiol 58:1904–1912

Tan GL, Shu WS, Hallberg KB, Li F, Lan CY, Huang LN (2007) Cultivation-dependent and cultivation-independent characterization of the microbial community in acid mine drainage associated with acidic Pb/Zn mine tailings at Lechang, Guangdong, China. FEMS Microbiol Ecol 59:118–126

Tsai YL, Olson BH (1991) Rapid method for direct extraction of DNA from soil and sediments. Appl Environ Microbiol 57:1070–1074

van de Vossenberg JLCM, Driessen AJM, Zillig W, Konings WN (1998) Bioenergetics and cytoplasmic membrane stability of the extremely acidophilic, thermophilic archaeon *Picrophilus oshimae*. Extremophiles 2:67–74

Wielinga B, Lucy JK, Moore JN, Seastone OF, Gannon JE (1999) Microbiological and geochemical characterization of fluvially deposited sulfidic mine tailings. Appl Environ Microbiol 65:1548–1555

Yang B, Shu WS, Ye ZH, Lan CY, Wong MH (2003) Growth and metal accumulation in vetiver and two Sesbania species on lead/zinc mine tailings. Chemosphere 5:1593–1600