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Effects of heap-bioleaching plant on microbial community of the nearby river

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ABSTRACT

The Zijinshan heap-bioleaching plant started the operation by the end of 2005, since the proximity of Ting River, concerns rose about the migration of acidophiles into this river. In this research, 72 liquid samples and 9 ore heap samples were collected, the distribution of acidophiles in Ting River were investigated by clone libraries, four key acidophiles *Acidithiobacillus*, *Leptospirillum*, *Sulfobacillus* and *Ferroplasma* were assayed by real-time polymerase chain reaction. The clone libraries results showed that 1291 OTUs were got from 3669 sequences, and these 1291 OTUs belonged to 16 phyla and 166 genera. The microbial community results indicated river water was fresh in 2009 relatively, nitrate and nitrite pollution might exist in 2012 and 2013, but the effect of heap-bioleaching plant on microbial community of the nearby river decreased from 2013. The shared OTUs results indicated the samples of 2012 had more acidophiles kinds, and the real-time PCR results indicated the key bioleaching microorganisms increased from 2009 to 2012, and decreased from 2012 to 2013 on the whole. The acid water leakage and pollution incident in 2012 had some effect on the river microbial community, and the microbial ecology of the river was in the remediation process in 2013.

1. Introduction

Bioleaching: a microbial process of metal recovery (Behera et al., 2011; Mishra et al., 2005) which was more suitable for the low grade ore especially (Ilyas et al., 2012; Pal et al., 2010). Bioleaching had many obvious advantages, such as the process was simple, the pollution and the energy consumption were less than the traditional methods (Pradhan et al., 2008). In China, the Zijinshan Copper Mine was the first commercial heap-bioleaching plant, which with a capacity of 10,000 t Cu/a in the end of 2005 (Ruan et al., 2006).

So far, a large number of papers focused on bioleaching microorganisms and bioleaching efficiency were published (Abdollahi et al., 2015; Dong et al., 2013; Li et al., 2013b; Lors et al., 2009; Rawlings, 1996; Vakylabad et al., 2011; Watling et al., 2014; Xie et al., 2007), but few research carried out on the subject of bioleaching microorganisms diffusion to the surrounding environment. A lot of researches have been carried out on the subject of bioleaching microorganisms' diffusion in our laboratory from 2009, and some promising results were also obtained from the recent researches.

The application of molecular biology techniques, especially 16S rRNA clone library (Kauppi et al., 2011) and real-time PCR (Lu et al., 2011), has progressed significantly in the field of microbial ecology. In this paper, we researched the effects of Zijinshan bioleaching system on the microbial community of the nearby river.

2. Materials and methods

2.1. Sites information

Zijinshan Copper Mine and Ting River located in Fujian province, belongs to subtropical maritime monsoon climate, has abundant rainfall and long frost free period all the year. The average temperature is 18.7–21.0, the average rainfall is 1031–1369 mm, these are suitable for tropical crops and tree growth.

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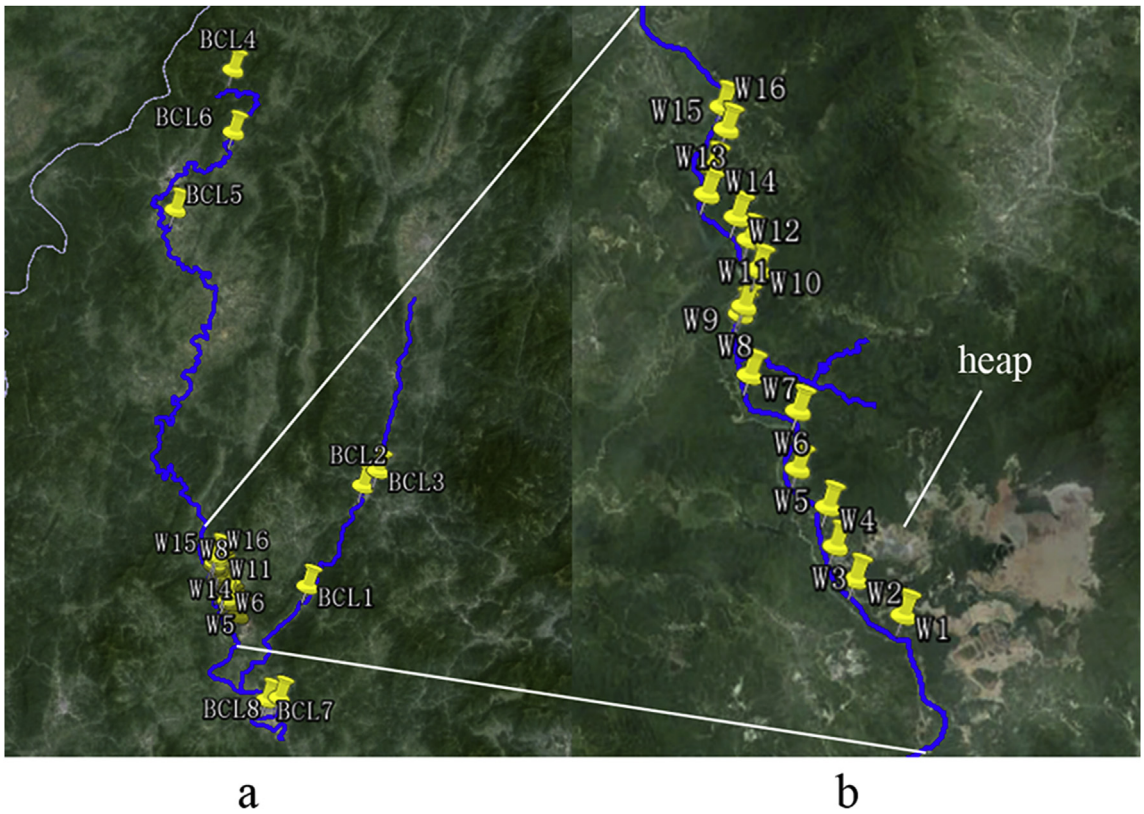

Fig. 1. Samples collecting sites.

2.2. Samples preparation

A total of 72 liquid samples (W1-W16 and BCL1-BCL8, 24 samples every year) were collected at the same sites in 2009, 2012 and 2013 respectively, the spatial distribution of sampling sites were shown in Fig. 1. Furthermore, 9 solid samples were collected from ore heap.

2.3. DNA extraction and sequencing

A bead-beating method (Oved et al., 2001) was used for the extraction of total DNA from soil samples. For construction of the bacteria clone library, 16S rRNA genes were amplified by polymerase chain reaction (PCR) with general primers (27f and 1492r) (Lane, 1991; Liang et al., 2014; Pinar et al., 2013) as primers, and the extracted total DNA as template. The amplified 16S rRNA genes were inserted into pGEM-T easy clone vectors (Promega, WI, USA) and transformed into *E. coli* JM109, these process referenced with corresponding operation manual respectively. At least 100 clones from each clone library were sequenced by Sangon Corp method (Shanghai, China). The representative 16S reads were deposited into NCBI GenBank under the submission ID KX010313-KX010417.

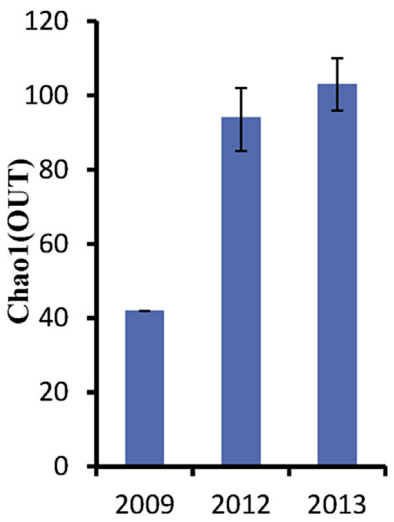

Fig. 2. Microbial richness index.

2.4. Bioinformatics analysis of community succession and function

The 16S rRNA gene of clone libraries were processed using the open-source program, Mothur v.1.33.3 (O'Brien et al., 2013; Schloss et al., 2009). Sequences were aligned with the SILVA database. The bad sequences were screened by screen seqs command with optimize = start and criteria = 95. Chimeras were removed using the chimera. uchime command (Edgar et al., 2011). The taxonomy of sequences were determined using the classify seqs command with trainset 9_032012, a cut-off of 80% and processors = 2. Distance matrices were generated using a cutoff of 0.30. Shared files were used to describe the dissimilarity among all samples. The diversity of OTUs and community overlap were also calculated using rarefaction analysis and Venn diagrams (Silveira et al., 2011).

2.5. Real-time PCR quantitative analysis of samples microorganisms

Real-time PCR based on SybrGreen I was performed with Rotor-Gene 6000 (Corbett Research). The PCR process referenced with SybrGreen PCR Master Mix operation manual (Applied Biosystems).

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