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Changes in the composition of an acid mine drainage microbial community upon successive transfers in medium containing low-grade copper sulfide

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ABSTRACT

A consortium of microorganisms from acid mine drainage samples was cultured in modified 9 K medium containing low-grade copper sulfide. The culture was maintained for sixty days and then transferred to fresh medium. This process was repeated three more times and a final consortium exhibiting a copper extraction rate of 89.3% was obtained. RFLP and microarrays analysis of 16S rRNA sequences retrieved from the consortia showed that *Acidithiobacillus caldus*, *Leptospirillum ferriphilum*, *Sulfobacillus* sp., *Acidiphilium* sp., and *Sulfolobus* spp. were represented in higher numbers in the consortia obtained in the copper-containing medium than in the original consortium. In contrast, a decrease in *Acidithiobacillus ferrooxidans*, *Alicyclobacillus* sp., *Pseudomonas* sp., and *Sulfobacillus thermosulfidooxidans* was observed. The abundance of genes related to sulfur metabolism from *At. caldus* and *Sulfolobus* spp., iron oxidation from *Leptospirillum* sp. and metal resistance from most of the detected microorganisms increased as the consortium was successively transferred into fresh medium.

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1. Introduction

Acid mine drainage (AMD) is generated by chemical and biological oxidation of copper sulfide, chalcopyrite, pyrite and other metal sulfides in mine waste heaps or in tailings from sulfide ore processing. Many microorganisms, including bacteria, eukaryota, and archaea inhabit such environments (Baker and Banfield, 2003). Studies have shown that different AMD systems differ in structure and diversity of their microbial communities due to variations in environmental factors such as temperature, ionic strength, and pH (Hugenholtz et al., 1998; Bond et al., 2000; Orphan et al., 2000; Yin et al., 2008). Some of these microorganisms including iron-oxidizers, sulfur-oxidizers, and heterotrophic acidophiles have been isolated and used as effective tools for extracting metals from low-grade or difficult-to-processes sulfide ores in the bioleaching industry (Rawlings et al., 1999; Rawlings, 2005; Johnson, 2008).

Bioleaching of low-grade primary copper sulfides is of interest to the industry because they are an abundant source of copper; however, the microorganisms involved in bioleaching are mainly acidophilic chemolithotrophic species which grow slowly and therefore achieve only low metal extraction (Stott et al., 2000; Watling, 2006). The interactions of different groups of bioleaching microorganisms among each other and with their environment also influence sulfide dissolution (Galleguillosa et al., 2008). Therefore, understanding the microbial ecology of bioleaching systems is a prerequisite for the development of more effective mineral-oxidizing consortia and enhanced leaching (Qiu et al., 2008).

Such consortia can be developed by adaptation of an initial microbial inoculum to a specific ore over time. In the current study, the composition of an initial inoculum and that of an adapted consortium were analyzed by restriction fragment length polymorphisms of 16S rRNA gene sequences. Changes in the abundance of acidophilic bacteria during the adaptation process relative to their initial abundance were determined by microarray analysis. The abundance of genes important to bioleaching was analyzed using a gene array.

2. Methods

2.1. Mineral components

Low-grade copper sulfide with a grain diameter of less than 75 μm was collected from Heilongjiang Province, China. The mineral sample contained about 0.38% chalcopyrite, 0.12% chalcocite, 0.006% free copper oxide, 0.004% combined copper oxide and 4.88% calcium carbonate as determined by X-ray diffraction.

2.2. Adaptation of microbial consortium

Acid mine drainage (AMD) samples from several copper mines in China were collected, the number of bacteria in each sample

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was determined by a Thomas counting chamber with an optical microscope (100×), and 10^9 microorganisms from each AMD sample were combined. The AMD mixture was subjected to centrifugation at 10,000*g* for 5 min, and the cell pellet was washed twice with distilled water adjusted to pH 2.0 with hydrochloric acid. Microbial consortium adaptation experiments were carried out in triplicate using 500 ml shake flasks containing 100 ml of 9 K basal salt medium (Silverman and Lundgren, 1959) without ferrous sulfate medium and with 5% (w/v) copper sulfide. The medium was inoculated with 10^7 cells/ml (consortium A) and incubation was carried out with shaking at 170 rpm. The microbial consortium was transferred to a new liquid medium on days 60, 120, 180, and 240, generating consortia B, C, D, and E. During the adaptation experiments, each consortium was everyday incubated at different temperatures of 25, 35, and 45 °C, successively (i.e., 25 °C from 0 to 8 h, 35 °C from 8 to 16 h and 45 °C from 16 to 24 h). This was applied because it may lead to a relatively rich bioleaching community. Hydrochloric acid or sodium hydroxide (10 mM) was added by hand every three days to keep pH value around 2.0 during the adaptation process. Hydrochloric acid was used instead of sulfuric acid since sulfuric acid may interfere with the measurements of elemental sulfur concentration in the leaching solution. Distilled water was added to flasks in order to compensate for evaporation losses. Uninoculated medium was used as abiotic controls.

2.3. Analytical techniques

Free bacteria in suspension were counted by direct counting, using a Thomas counting chamber with an optical microscope (100×). The levels of the ions in solution were determined by Inductive Coupled Plasma Emission Spectrometry. The ferrous ion concentration was determined by titration with potassium dichromate ($K_2Cr_2O_7$). The pH of the leaching systems was measured with a pH meter. For all of the experiments distilled water was used.

2.4. DNA extraction and purification

Suspended and attached cells were collected as described by Zeng et al. (2010). DNA from the initial consortium (consortium

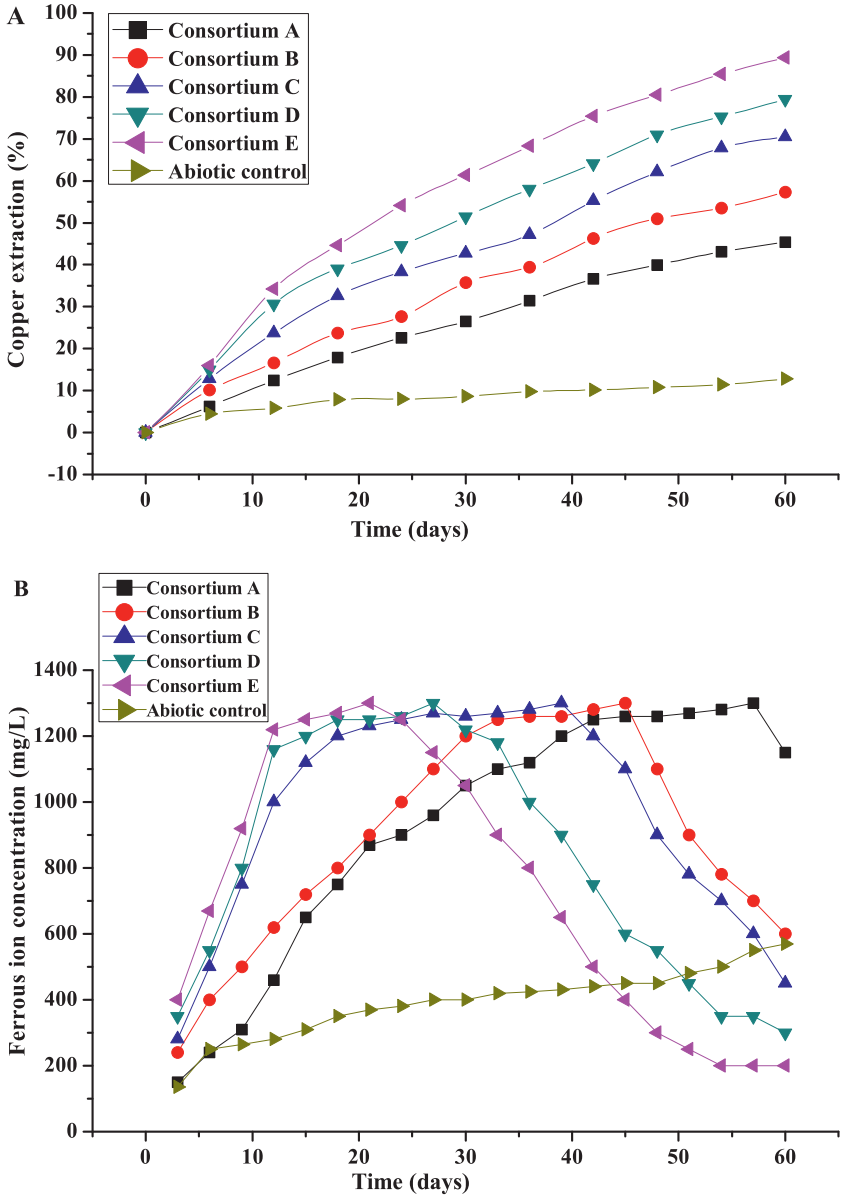

Fig. 1. (A) Copper extraction by consortia A–E. (B) Ferrous ion concentration in solution in the presence of consortia A–E.

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