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Original article

Comparative metagenomics reveals microbial community differentiation in a biological heap leaching system

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Abstract

The microbial community in a biological heap leaching (BHL) system is crucial for the decomposition of ores. However, the microbial community structure and functional differentiation in different parts of a biological heap leaching system are still unknown. In this study, metagenomic sequencing was used to fully illuminate the microbial community differentiation in the pregnant leach solution (PLS) and leaching heap (LH) of a BHL system. Long-read sequences (1.3 million) were obtained for the two samples, and the MG_RAST server was used to perform further analysis. The taxa analysis results indicated that the dominant genera of PLS is autotrophic bacterium *Acidithiobacillus*, but heterotrophic bacterium *Acidiphilium* is predominant in LH. Furthermore, functional annotation and hierarchical comparison with different reference samples showed that the abundant presence of genes was involved in transposition, DNA repair and heavy metal transport. The sequences related to transposase, which is important for the survival of the organism in the hostile environment, were both mainly classified into *Acidiphilium* for PLS and LH. These results indicated that not only autotrophic bacteria such as *Acidithiobacillus*, but also heterotrophic bacteria such as *Acidiphilium*, were essential participants in the bioleaching process. This new meta-view research will further facilitate the effective application of bioleaching.

Keywords: Metagenomics; Biological heap leaching system; Microbial community; KEGG; MG-RAST server

1. Introduction

Biohydrometallurgy is increasingly becoming a cause for concern because it can effectively utilize low-grade resources to expand copper resource reserves in existing resources lacking copper [1]. Biological heap leaching (BHL) is the most widespread among several applications in biohydrometallurgy, and its main advantages are its low cost, ease of use and limited pollution. However, its long production cycle is the greatest disadvantage of the BHL process [2]. The microorganism community is crucial for the efficiency of production of a BLH system [3–5]. Therefore, it is crucial to elucidate microorganism community structure and function for enhancing the production efficiency of the BLH system.

The Jiangxi Copper Corporation (JCC), established in 1979, is now the largest copper producer and copper manufacturer in China. The BHL system of the Dexing copper mine has successfully run since 1995. In recent years, studies have assessed the microbial community in the Dexing BHL system using different analysis methods, such as restriction fragment length polymorphism (RFLP) [6] and microarrays [7,8], which mostly focused on acid mine drainage. These studies showed that the drainage is dominated by bacteria rather than archaea, with the major phyla represented by *Acidobacteria*, *Actinobacteria*, *Nitrospira*, *α-Proteobacteria*, *γ-Proteobacteria*,

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Firmicutes and *δ-Proteobacteria*. Most of these chemolithotrophic acidophiles often contribute to iron and sulfur cycling via oxidization of reduced inorganic sulfur and ferrous iron compounds [9]. However, because these studies assessed microbial diversity simply based on 16S rRNA gene or gyrB gene analysis, they are insufficient to provide an in-depth view of the microorganism community structure or the ecologically relevant genes involved in various metabolism pathways. Until now, no research has been conducted regarding microorganism community differentiation between pregnant leach solutions (PLS) and the leaching heap (LH) in the BLH system of the Dexing copper mine.

Metagenomics has been defined as a function-based or sequence-based cultivation-independent analysis of the collective microbial genomes present in an environment [10]. As a result of the rapid development of high throughput sequencing, metagenomic analysis became a more feasible technology to explore microbial community structure and function in depth. In a number of studies, various standard methods, such as reads assembling, local alignments against different databases and associating best hits to different classifications, were employed to analyze the taxonomic and functional classification of metagenomic data [11,12]; however, a more comprehensive view of the microbial community can be obtained by directly using unassembled reads assignment [13]. Furthermore, because the sequence lengths of reads from a Roche 454 GS FLX sequencing plate, which was employed in this investigation, were mostly between 200 bp and 700 bp, these unassembled reads datasets possessed bioinformatics significance and were directly analyzed.

In this work, the microbial community differences between the leaching heap (LH) and the pregnant leach solution (PLS) of the Dexing copper mine were analyzed using whole genome sequencing (WGS) metagenomic strategy and GenBank, RefSeq and SEED databases. The reads was also analyzed against clusters of orthologous groups (COG) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases to obtain a more accurate view of the genes present in and the functional differentiation of the BHL system. Additionally, the community functions of PLS and LH were compared with four other reference metagenomic samples for researching the community peculiarity of two target samples.

2. Materials and methods

2.1. Sample collection and metagenomic DNA extraction

The BHL system of the Dexing copper mine was located at 29° 00′ 26.92″ N and 117° 42′ 29.87″ E in the Jiangxi Province, China (Fig. S1). The run model of this system is shown using red lines in Fig. S1. The ore samples were obtained from five different sites, which were 1.5 m below the surface of the heap. Each ore sample was approximately 50 kg. The solution samples were collected from five sites around the pregnant leach solution pool, and the volume of each sample was roughly 25 L. All of the ore samples were absolutely mixed; then, 50 kg was removed from each sample and named LH. LH was washed by a sterile 9K medium (pH 2.0, volume 50 L) and organisms were obtained from the ore surface in the solution. All of the solution samples were also mixed, and 50 L was removed; the removed portion was named PLS.

First, the PLS sample and the eluant of the LH sample were filtered through qualitative filter paper to remove particles and large cells. Then, to filter the previously filtered solution, the microorganisms in two samples were obtained using a vacuum filtration device with a 0.22-μm filter membrane. Metagenomic DNA was extracted from the microorganisms on the filter paper according to the method of Jizhong Zhou [14] and then resuspended in TE buffer. The DNA solution was analyzed by agarose gel (1%, containing ethidium bromide) electrophoresis.

2.2. Pyrosequencing and sequence treatment

A total of 15 μg of metagenomic DNA for each sample was used for library preparation using emulsion PCR, and the metagenomic sequencing work was performed on a Roche 454 GS FLX Titanium System (Majorbio, China). Sequence data were sorted into each sample batch using a barcode tag. The raw reads were filtered to obtain high-quality clean reads by removing the primer sequences, bar codes and reads containing ambiguous bases or homologous using the sequence analysis pipeline of MOTHUR software (sffinfo and trim.seqs commands, http://www.mothur.org/wiki/sequence_processing). Sequences with a minimum length of 100 bp were evaluated using a sliding window of 20 bp; only those sequences with a recommended quality score of $\geq$20 were retained [15].

2.3. Taxonomic assignment and functional annotation of metagenomic sequences

After quality assessment and trimming using MOTHUR software, the retained sequences were uploaded onto the MG-RAST server (http://metagenomics.anl.gov) under project IDs 4554868.3 (PLS) and 4554867.3 (LH). The main pipeline was shown as follows. First, all uploaded sequences were scanned by quality control (QC). Ribosomal RNA genes and ORFs were predicted in sequence datasets that passed QC. Furthermore, for analyzing the taxonomic assignments of the samples, all sequences that passed QC were annotated to the indicated taxonomic level against GenBank, RefSeq and SEED databases. Conversely, to indicate the functional categories of samples, all ORFs were annotated using a public main database, such as the COG and KEGG databases. Finally, microorganism community structure and functional category were defined based on the assigned annotation.

In detail, the taxonomic assignment was performed using BLAST on the MG-RAST server against the corresponding databases with a cut-off E-value of 1e−10 and a minimum alignment size of 50 bp. For microbial community function aspects, all ORFs were annotated using BLAST (cut-off E-value of 1e−10 and minimum alignment of 15 bp) against COG and KEGG databases. All KO numbers of the two samples were recorded for further analysis.

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