P17

Microbial population structure analysis of Tsukahara hot spring in Oita and search for effective microbes for biomining and bioremediation

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Abstract

This study investigated the microbial population structure in Tsukahara hot spring located in Oita. Extremely acidophilic microbes belonging to genera such as *Acidianus*, *Metallosphaera*, *Ferroplasma*, *Leptospirillum*, *Sulfobacillus*, and *Acidithiobacillus* were shown to be present at the crater and downstream of the source, which are widely utilized for biomining. Furthermore, by enrichment cultivation for isolation of effective microbes, extremely acidophilic, Fe(II)- and/or sulfur-oxidizing microbes showing high As(III)-tolerance were cultivated. This result suggested these microbes could be applied to biomining and bioremediation related to highly toxic arsenic.

1. Introduction

Development of biological techniques for mining and environmental remediation has been proceeding. Actually we have previously conducted biooxidation of arsenic-containing refractory gold ore concentration using moderately thermophilic, extremely acidophilic bacteria (45°C), and it was shown to be one of the most effective pre-treatment options compared with other abiotic pre-treatment approaches (Tanaka et al., 2015). Moreover, we have successfully crystallized bio-scorodite from synthetic copper refinery wastewater containing arsenite (H_3AsO_3; As(III)) by utilizing thermo-acidophilic iron-oxidizing archaeon, *Ac. brierleyi* (70°C) (Okibe et al., 2014). For further development of biological techniques for applying to mining and environmental remediation, it has possibility to utilize the new extremely acidophilic, iron- or sulfur-oxidizing microorganisms.

Tsukahara hot spring located in Oita is known as an extreme environment with its highly acidic pH and highest Fe ion concentration in Japan. In this extreme environment, thermophilic and acidophilic Fe-oxidizing microbes and Fe-reducing microbes may exist. Their Fe-oxidation ability can be used for biomining, such as bioleaching and biooxidation (Cu, Au), and bioremediation (As). Fe-reduction ability for bioremediation of toxic metals (Cr, V) and biorecovery of valuable metals (Pd, Pt).

Additionally, in metallurgical operations, arsenic (especially in the form of As(III)) is a major impurity contained in low-grade copper refractory ores and its process solutions. As(III) is known to be highly toxic and mobile (Matschullat, 2000). Therefore, iron- and sulfur-oxidizing microbes possessing high As(III)-tolerance or oxidizing ability would have advantages.

In this research, in order to enrich and isolate useful microorganisms from the sample taken at Tsukahara hot spring to apply them to biomining application, microbial population dynamics analysis by cycle sequencing and enrichment cultivation of Fe(II)- or sulfur-oxidizing microbes having As(III)-tolerance or oxidizing ability were conducted.

2. Materials and Methods

2.1. Sampling

Water and soil samples from the crater around the source, water and biofilm samples from downstream of the source, and black sediment samples around the fumarole were collected. Water pH was 1.4 in both crater and downstream, and temperature at crater, downstream, and fumarole were 30°C, 50°C, and 99°C, respectively.

2.2. Microbial population dynamics analysis by cycle sequencing

Cycle sequencing identifies the

microbes by comparing their 16S rRNA gene sequence with those from known microbes on the database. Genome DNAs were extracted from the samples, 16S rRNA genes were amplified by polymerase chain reaction (PCR), cloning was carried out using *E. coli* host cells, and the sequencing was conducted using 3130xl Genetic Analyzer (Applied Biotechnologies, Life Technologies). Finally the obtained sequences were compared to those on database GenBank using BLAST research.

2.3. Enrichment cultibation

For enrichment cultivation of Fe(II)- or sulfur-oxidizing microbes, water and soil samples from crater around the source and biofilm samples from downstream of the source were inoculated in 300 ml flasks containing 100 ml of heterotrophic basal salts medium (pH 1.5) with 10 mM Fe(II) (for Fe(II)-oxidizing microbes), 0.1% (w/v) elemental sulfur (for sulfur-oxidizing microbes) and 0.02% (w/v) yeast extract. Furthermore, to cultivate the microbes having As(III)-torelance or oxidizing ability, 1-13 mM As(III) was supplemented in stages. Flasks were incubated at 45 or 60°C, shaken at 150 rpm. Samples were regularly taken to monitor pH, Eh (vs SHE), cell density, and concentrations of Fe(II) and As(III).

3. Results and Discussion

3.1. Microbial population dynamics

In the crater and downstream of the source, the extremely acidophilic microbes belonging to genera such as *Acidianus*, *Metallosphaera*, *Ferroplasma* (archaea), and *Leptospirillum*, *Acidithiobacillus,Sulfobacillus* (bacteria), were shown to be present. These extreme acidophiles are widely used for bioleaching and biooxidation. Therefore, it is possible to use them for biomining and bioremediation if these microbes are to be isolated from this extreme environment.

In addition, 16S rRNA gene homologies between the microbes detected in this study and known isolates were only 80-90%. Therefore it is possible that novel microbes are present in this environment.

3.2. Enrichment cultivation

Enrichment cultivation using Fe(II) or elemental sulfur was repeated several times, and Fe(II) and elemental sulfur were oxidized readily in all cultures. However, when 13 mM As(III) was added to the medium, sulfur oxidation was not observed in the culture from crater soil (incubated at 45 and 60°C), and biofilm in downstream of the source (incubated at 45°C). By contrast, Fe(II) oxidation in the culture for crater soil and biofilm (incubated at both 45 and 60°C), and elemental sulfur oxidation in the culture for biofilm (incubated at 60°C) were observed even in the presence of 13 mM As(III). This results indicated that the extremely acidophilic, Fe(II)- or sulfur-oxidizing microbes having high As(III)-tolerance were cultivated.

4. Conclusion

The extremely acidophilic microbes such as *Acidianus*, *Metallosphaera*, *Ferroplasma*, *Leptospirillum*, *Sulfobacillus,* and *Acidithiobacillus*, which were present at downstream of the source and the crater, are generally used for bioleaching and biooxidation. Moreover, it was found that the extremely acidophilic, Fe(II)- or sulfur-oxidizing microbes showed high As(III)-tolerance by enrichment cultivation. Therefore, it is possible to utilize them for biomining and bioremediation related to highly toxic arsenic if they can be isolated from this extreme environment. For future studies, isolation and identification of useful microbes is currently being carried out.

Reference

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