

Principal Research Results

High Density Cultivation of Cr(VI) Reducing Bacteria using Electrochemical Accumulation

Background

There are many kinds of bacteria in nature that have special metabolisms like metal detoxification activities. Although those bacteria are expected to be utilized for bio-remediation to clean up contaminated soils, ground waters and drains, almost of all of them are categorized as “uncultured bacterium” which cannot be cultivated by any previous cultivation styles, and this difficulty culture inhibits their practical use in industrial plants or reactors. A Cr(VI) reducing bacterium is one of these bacteria.

We have been developing a growth enhancement of previously uncultured microorganisms by electrochemical reactions. It is supposed that the Cr(VI) reducing bacteria can be accumulated by the electrochemical cultivation.

Objectives

To clarify the optimum culture condition and to probe availability of electrochemical accumulation of Cr(VI) reducing bacteria.

Principal Results

1. Growth of Cr(VI) reducing bacteria under a conventional culture method.

Cr(VI) reducing bacteria were collected from an environmental soil after 6 month cultivation under an anaerobic medium condition with 0.2 mM of Cr(VI) ions which inhibited almost all the bacteria's growth except for Cr(VI) reducing bacteria (Fig.1). After several subcultures, the Cr(VI) reducing bacteria had adopted and acquired ability that 90% of Cr(VI) ions in the medium were reduced to Cr(III) ions within 1 week. However, obtaining pure cultivation of the Cr(VI) reducing bacteria using conventional method was difficult because as the concentration of Cr(VI) ions decreases, dominant bacteria from the original inoculation begin to thrive. It was suggested that maintenance of Cr(VI) ions in the medium was required for selective accumulation of Cr(VI) reducing bacteria.

2. Accumulation of Cr(VI) reducing bacteria using an electrochemical technique.

To probe an accumulation of Cr(VI) reducing bacteria, electrochemical cultivation system was constructed to maintain Cr(VI) ions during the cultivation (Fig.2). The electrochemical bath was constructed, consisting of a cultivation side with Pt anode and a counter side with carbon cathode separated by an ion exchange membrane. After 2 weeks cultivation of the Cr(VI) reducing consortium in a medium including 0.1 mM of Cr(VI) ions applying 1.0 V (vs. Ag/AgCl reference) of potential, only one species of bacteria grew selectively, in contrast to at least three types of strains that appeared in a medium of conventional cultivation without electrolysis (Fig.3).

From these results it is concluded that the electrochemical cultivation is a useful method to accumulate Cr(VI) reducing bacteria from a mixture of microorganisms. The electrochemical accumulation also might be applicable to the growth of other uncultured metal reducing bacteria.

Future Developments

Applicability of the electrochemical cultivation method will be investigated using other metal reducing bacteria.

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Reference

N. Matsumoto, et.al., 2005, “Electrochemical control of bacteria (Part 8). Acquisition of a chromium reducing bacterium and its accumulation using electrochemical cultivation”, Technical Report V04006 (in Japanese)

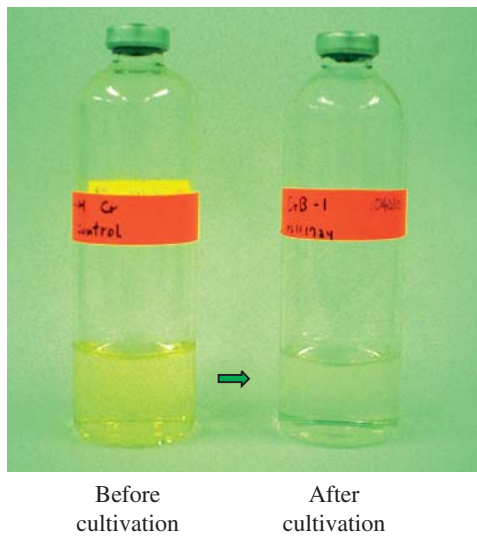


Fig.1 Reduction of Cr(VI) ions by Cr(VI) reducing bacteria

Cr(VI) ions showed yellow color in the medium (left) reduced to Cr(III) by Cr(VI) reducing bacteria and the color disappeared due to its precipitation (right).

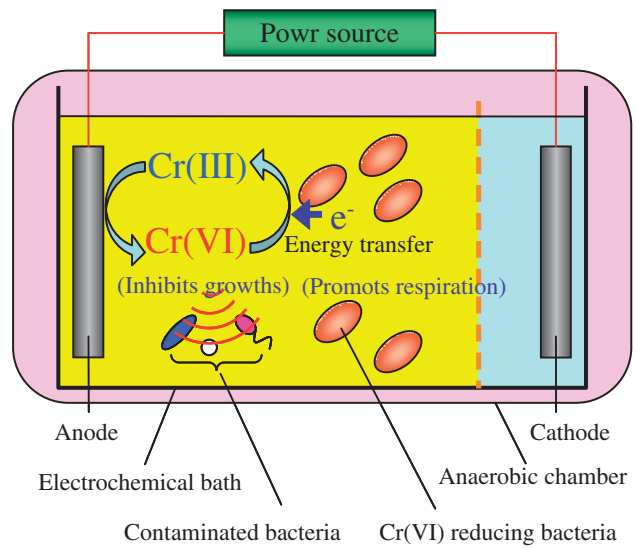
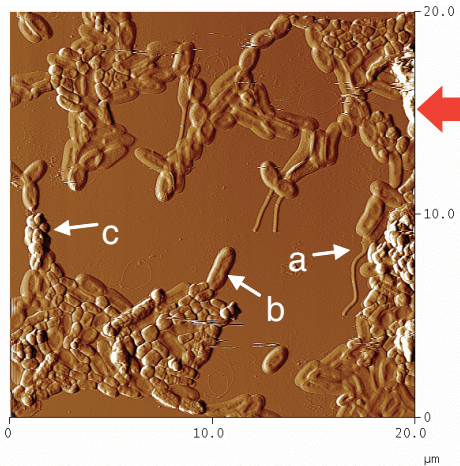


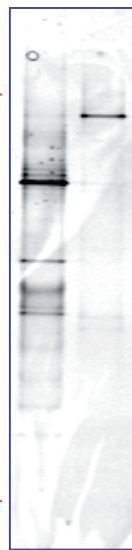
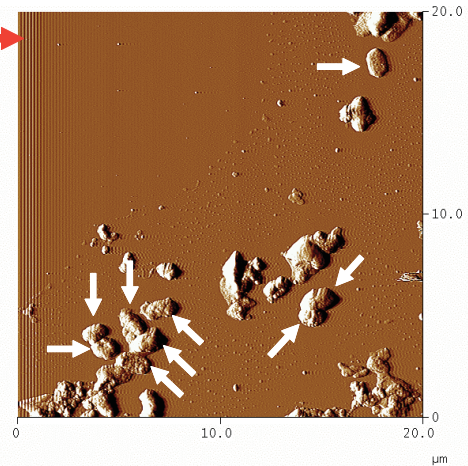
Fig.2 Electrochemical cultivation system for accumulation of Cr(VI) reducing bacteria

Cr(VI) ions generated on the anode shows toxicity to contaminated bacteria to inhibit their growth. On the other hand, it promotes respiration of Cr(VI) reducing bacteria.

Bacteria grown by conventional cultivation



Bacteria grown by electrochemical cultivation



Result of DNA analysis (DGGE* method)

Fig.3 Confirmation of accumulation of Cr(VI) reducing bacteria by electrochemical cultivation

Only one species of bacteria was observed in a medium with electrolysis (right image, arrows), in contrast to at least three types of strains appearing in a medium without electrolysis (left image, a - c). In addition, a DNA analysis supported that bacteria grown by electrochemical cultivation were uniform (center image).

*DGGE: Denaturing Gradient Gel Electrophoresis. Number of bands shown in DGGE represents the number of species of bacteria existing in the medium.