Characterization of Fe (III)-reducing enrichment culture and isolation of Fe (III)-reducing bacterium Enterobacter sp. L6 from marine sediment

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To enrich the Fe (III)-reducing bacteria, sludge from marine sediment was inoculated into the medium using Fe (OH)₃ as the sole electron acceptor. Efficiency of Fe (III) reduction and composition of Fe (III)-reducing enrichment culture were analyzed. The results indicated that the Fe (III)-reducing enrichment culture with the dominant bacteria relating to Clostridium and Enterobacter sp. had high Fe (III) reduction of (2.73 ± 0.13) mmol/L Fe (II). A new Fe (III)-reducing bacterium was isolated from the Fe (III)-reducing enrichment culture and identified as Enterobacter sp. L6 by 16S rRNA gene sequence analysis. The Fe (III)-reducing ability of strain L6 under different culture conditions was investigated. The results indicated that strain L6 had high Fe (III)-reducing activity using glucose and pyruvate as carbon sources. Strain L6 could reduce Fe (III) at the range of NaCl concentrations tested and had the highest Fe (III) reduction of (4.63 ± 0.27) mmol/L Fe (II) at a NaCl concentration of 4 g/L. This strain L6 could reduce Fe (III) with unique properties in adaptability to salt variation, which indicated that it can be used as a model organism to study Fe (III)-reducing activity isolated from marine environment.

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Microbial dissimilatory Fe (III) reduction is a process that can cause the release of soluble Fe (II) by coupling the oxidation of organic matter to the reduction of Fe (III) (1). The process plays an important role in the geochemical cycling of iron and organic matter in anoxic ecology systems (2,3). The organisms capable of coupling the oxidation of organic matter to the dissimilatory reduction of Fe (III) have been described as Fe (III)-reducing bacteria. Dissimilatory Fe (III) reduction may occur during the respiratory, fermentative or photosynthetic metabolism of various Fe (III)-reducing bacteria (4–6). These bacteria could transfer electrons derived from the oxidation of organic matter to carbon dioxide with Fe (II) as the sole electron acceptor (7).

Microbial Fe (III) reduction can contribute to purification of pollutants and has the application of environmental protection (8,9). Geobacter metallireducens GS-15 was the first reported Fe (III)-reducing bacterium, which could simultaneously decompose benzene, toluene and other aromatic compounds (10). Iwahori et al. (11) demonstrated that Fe (III)-reducing microbial enrichment cultures had the ability of removal of toxic metal cations from water. Zhou et al. (12) reported that Klebsiella sp. strain FD-3 could reduce Fe (III) EDTA and remove NOx efficiently. The Fe (III)-reducing bacteria are being increasingly recognized as an ecological and environmental important group of microorganisms (13). Many Fe (III)-reducing bacteria were isolated and had been reported for significant contribution to iron and organic matter cycling in fresh condition (14–16). However, few studies have been reported on Fe (III) reduction by Fe (III)-reducing bacteria from the marine sediments.

The marine sediments with increasing depth below the seafloor have been the important habitats for the Fe (III)-reducing bacteria due to the special anaerobic environment. Dissimilatory Fe (III) reduction is the most reaction among a series of microbial-mediated redox reactions occurs in the marine sediments (17,18). Bohai is one of the inland seas in China. Due to the rapid development in coastal regions, Bohai has been subjected to both heavy metal and organic contamination. These pollutants will be eventually accumulated in submarine sediments. Fe (III)-reducing bacteria can change the formation of insoluble Fe (III) to the soluble Fe (II), which influence the distribution of toxic trace metals and strengthen the liquidity of pollutants in the sediments. Therefore, Fe (III)-reducing bacteria using Fe (III) as the most potentially electron acceptors in marine sediments will provide an alternative approach for the purification of the contaminants.

In this work, by using sediment from Bohai Sea, China, we determined the efficiency of Fe (III) reduction and composition of microbial community of the Fe (III)-reducing enrichment culture. In the enrichment culture, a new Fe (III)-reducing bacterium was isolated. The level of microbial Fe (III) reduction by the isolated strain was optimized under different carbon sources and salt concentration conditions.

MATERIALS AND METHODS

Source of the organism The marine sediment was collected from Bohai Sea, in Tianjin, China (longitude of 116 and latitude of 38). The sediment sample was taken from approximately 15 m below the sea surface and placed in sterile plastic
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RESULTS AND DISCUSSION

Fe (III) reduction by enrichment culture After three times of enrichment procedure, cell growth and Fe (III)-reducing activity of the enrichment culture with different Fe (III) concentration, 0, 4, 8, 11 and 15 mmol/L, respectively were investigated (Fig. 1). Bacterial growth of the mixed culture was significantly stimulated by the addition of Fe (III) as an electron acceptor and sensitive to the supplement of different Fe (III) concentrations in the medium. The maximum optical absorbance (1.4335 ± 0.12) and the highest Fe (III) conversion (2.73 ± 0.113) mmol-L^-1 Fe (II) were obtained by the Fe (III)-reducing enrichment culture inoculated in the medium with Fe (III) concentration of 8 mmol/L. The result was similar with the previous study that the bacteria could produce greater accumulations of Fe (II) with respect to the increased initial supply of Fe (III) during cell growth (21,22). There was a decrease on Fe (III) reduction with the increase of Fe (III) concentration above 8 mmol/L. At the Fe (III) concentration of 15 mmol/L, the significant decrease on cell growth and Fe (III) reduction was found. The addition of excess amount of Fe (III) may negatively impact the enrichment of Fe (III)-reducing bacteria.

Composition analysis of microbial community We used PCR-DGGE to determine composition of Fe (III)-reducing enrichment cultures. Fig. S1 shows the profile of the DGGE bands for the microbial community in the medium with Fe (OH)_3 of different concentrations (4, 8, 11 and 15 mmol/L). The major bands (B1, B2 and B3) in the DGGE gels were excised and purified to determine the sequence. The nucleotide sequences of the 16S rRNA gene of major bands have been deposited in GenBank, with accession number of KP278236, KP278237 and KP278238. The result indicated that the dominant bacteria inoculated with these three different Fe (III) concentrations (4, 8 and 11 mmol/L) were basically identical and closely related to Clostridium and Enterobacter species. It meant that the effect of Fe (III) concentration on Fe (III) reduction was microbial activity rather than composition of the microbial community. The major bands in the DGGE gels of the mixed culture enriched at Fe (III) concentration of 15 mmol/L were not formed. It meant that the number of bacteria enriched at this Fe (III) concentration was decreased significantly with the increasing of Fe (III) concentration. The cell growth of Fe (III)-reducing bacteria was inhibited by high Fe (III) concentration. The result was similar as the report that the excess amount of Fe (III) may lead to the formation of iron mineral coatings on cell surface of the Fe (III) reducing organisms, which may be unfavorable for cell growth and microbial activity (23).

Isolation and identification of the strain After five times of solid–liquid separation, a strain named as L6 was selected for the highest Fe (III)-reducing activity. The bacterial colonies by