Magnetite formation through thermophilic anaerobic nitrate-depending Fe(II) oxidization bacteria of Tibetan hot spring

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The Tibetan Plateau hosts thousands of hot springs, which are inhabited by various thermophilic microorganisms. For example, anaerobic nitrate-depdending Fe(II) oxidization bacteria (NDFOB) are known to contribute to iron biogeochemical cycling and mineralization. However, little is known about the NDFOB community composition and the process of nitrate-depending Fe(II) oxidization. In this study, sediments were collected from three neutral thermophilic hot springs of QZM-1, QZM-2 and QZM-16, with temperatures higher than 80 ^{o}C. NDFOB enrichment experiments were established with the collected hot spring sediments by supplementing ferrous iron [Fe(II)], nitrate and lactate, followed by construction of 16S rRNA gene clone library. Phylogenetic analysis showed that the NDFOB population was mainly affiliated with phyla of *Betaproteobacteria, Alphaproteobacteria* and *Firmicutes*. The kinetics of nitrate-depdending Fe(II) oxidization by the three NDFOB enrichments were investigated by adding Fe(II), NO_3^- and lactate in growth medium. During growth, visible black precipitation was produced within one week. The consumed NO_3^- and produced Fe(III) was approximately in the ratio of 1:4 and NO_2^- was detected as the intermediate product but did not accumulate, indicating that the NO_3^- may be reduced to N_2O or N_2 in the Fe(II) oxidization process. The amount of the consumed Fe(II) was higher than the produced Fe(III), indicating that part of Fe(II) was involved in the mineralization. X-ray diffraction (XRD) and scanning electron microscopy (SEM)-energy dispersive spectrometry (EDS) analysis showed the resulted precipitation mainly consisted of magnetite crystals with different morphology from nanoball to mature rhombic dodecahedrons or regular hexahedrons. These results together increased our understanding on NDFOB involved in the process of nitrate-depending Fe(II) oxidization and their roles in promoting iron and nitrate cycling and mineralization in geothermal ecosystems.

Keywords: Tibetan hot spring, anaerobic Nitrate-depending Fe(II) oxidization bacteria, magnetite , Betaproteobacteria, Alphaproteobacteria, Firmicutes

