

# Diversity of thermophilic sulfate-reducing prokaryotes in hydrothermal environments

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Little is known about the system of sulfur cycle under thermophilic and oxygen-limiting conditions such as deep-sea hydrothermal vent site terrestrial and hot spring, compared to the cycle observed in mesophilic coast and marine sediments. Sulfate-reducing prokaryotes (SRP), that use sulfate as an electron acceptor and product sulfide, are obligately anaerobic microorganisms. Several thermophilic sulfate-reducing bacteria (SRB) within the *Thermodesulforhabdus*, *Desulfotomaculum*, *Thermodesulfobacterium*, *Thermodesulfovibrio*, and sulfate-reducing archaea within the *Archaeoglobus* have been isolated from hydrothermal environments. However, little is known about the distribution, diversity, and role in the sulfur cycle of the thermophilic SRP.

The isotope record of microscopic sulfides shows the evidence for the biological sulfate reduction at 3.47 billion years old [Shen et al., 41:77-81, 2001, Nature]. The study on sulfur cycle by the microorganism in the deep-sea hydrothermal vent and terrestrial hot spring should provide the evidence for microbial sulfur cycle on early earth, because it has been hypothesized that the modern thermophilic and oxygen-limiting conditions are similar to the conditions on early earth, hotter and more anaerobic than it is now. In this study, for the understanding of the sulfur cycle under thermophilic and oxygen-limiting conditions, we analyzed the distribution and diversity of SRP in hydrothermal environments; deep-sea hydrothermal vent (Suiyo Seamount), terrestrial hydrothermal subsurface mine (Toyoha), and hot springs (Nakabusa and Yumata), with the PCR primer set for the dissimilatory sulfite reductase (DSR) gene [Wagner et al., 180:2975-2982, 1998, J. Bacteriol.].

In Suiyo Seamount, novel DSR genes were detected from three sampling sites as follows: A vent-catheter of stainless steel pipe [Higashi et al., in press, FEMS Microbiol. Ecol.], that placed on the hydrothermal vent (275C degree) and incubated during 3 days. Effluent water (4C degree) from casing pipe established with a tethered marine rock-drill (the so-called BMS: Benthic Multi-coring System). Diffuse flow (3 to 50C degree) from white patch area. The DSR genes were phylogenetically related to those of the thermophilic SRB *Thermodesulfobacteria*. In Toyoha mine, the phylogenetic analysis revealed that *Desulfotomaculum*-like DSR sequences dominated in the groundwater at sites-A (53C degree) and -B (66C degree), whereas *Thermodesulforhabdus*-like sequences dominated within microbial mats at sites-B and -D (73C degree). In Nakabusa and Yumata hot springs, *Thermodesulfobacteria*-like DSR genes were detected from submerged slime microbial streamer developed in spring water (72-80C degree, pH6.3-8.8). Moreover, with the analysis of microbial community structure based on 16S rRNA genes, it was demonstrated that *Thermodesulfobacteria*-like microbes were dominated in the microbial streamers. Based on the radioactive sulfate tracer technique, the sulfide production through sulfate-reduction by the streamers occurred. The biological sulfide production from the streamers was observed during incubation at in situ temperature.

The search and analysis of DSR gene obtained from deep-sea hydrothermal vent, hydrothermal subsurface mine, and terrestrial hot springs reveals that the DSR genes were markedly different from that of the known cultivated SRP, and formed the phylogenetically individual branch, respectively. It is essential to undertake the further study to clarify the system of sulfur cycle in hydrothermal environments.