Biological nitrogen fixation coupled to chemolithotrophic sulfur metabolisms in a thermophilic microbial community in a hot spring

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Thermophilic microbial communities are potent model systems for ecological and evolutionary analysis in ecosystems. One important physical component related to evolutionary and ecological dynamics in these systems is the availability of fixed nitrogen, which can only be acquired from the atmosphere (from N_2) by the action of nitrogenase, an enzyme which might have emerged as early as 3.5 billion years ago. Molecular based studies have suggested a distribution of nitrogen-fixing bacteria and archaea in hydrothermal vents and geothermal springs, however, the activities and ecological consequences of these metabolisms are poorly understood. Here, we detected and characterized nitrogen-fixing activity of chemosynthetic microbial communities developed at 74˚C at sulfidic and slightly alkaline hot spring water.

N_2-fixation (nitrogenase activity) was determined using the acetylene reduction assay. Collected microbial communities were incubated in hot spring water in a sealed vial in situ or at 70˚C in the laboratory. Nitrogenase activities were detected in conditions where moderate amounts of methane were produced, but not detected under conditions where methane production was at the observed maximum or minimum. These results suggest that nitrogen fixation occurs within a limited range of redox levels in the communities.

Addition of molybdate, an inhibitor of anaerobic sulfur metabolisms which have sulfite as an intermediate (e.g. sulfate reduction, and sulfur disproportionation), inhibited the nitrogenase activity of the communities. Dispersion of cell aggregates of the communities also decreased the nitrogenase activity, but the activity was partially recovered by amendment with H_2 and CO_2. These suggest that the nitrogenase activity in the communities is coupled with hydrogen-autotrophic and anaerobic sulfur metabolisms. In addition, cell aggregation may contribute to efficient interactions which support the currently unknown N_2-fixing microbe(s).
Taken together, our findings provide new insight into the ecological contributions of anoxic sulfur metabolisms in chemosynthetic thermophilic microbial communities.

Keywords: nitrogen fixation, sulfur metabolism, thermophiles, geothermal spring

