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Microbiology and geochemistry of subsurface geothermal stream in Hishikari Gold Mine, Japan

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Terrestrial hot spring environments support a diverse unique microbial community including thermophilic microorganisms with novel metabolic capacities. However, little is known about relation among microbial community structure, energy and carbon fluxes for subsurface ecosystem that occurs when subsurface geothermal aquifers emerge into oxic environments under dark conditions.

The Hishikari gold mine is located at the northwestern part of a volcanically active region near Mt. Kirishima in the Kagoshima prefecture, Japan. A subsurface volcanic hot aquifer (>72 degree Celsius) exists under the mine. In the deepest part of the mine tunnel (320m below land surface), a dense microbial community occurs at the point of emergence of a geothermal water stream which contains plentiful amount of hydrogen, methane and ammonium.

We have analyzed microbial community structure, energy flux and carbon flux by applying 16S rRNA gene clone analysis, whole cell FISH, competitive PCR (cPCR) for quantification of the key metabolic enzyme genes, and isotopic ratio of organic and inorganic compounds (e.g., ammonium, methane, carbon dioxide, whole cell, IPLs). 16S rRNA gene clone analysis shows that hydrogen and sulfur-oxidizing bacteria (*Sulfurihydrogenobium* sp.) and methane-oxidizing bacteria (*Methylothermus* sp.) has dominated in mat in the upper stream (72 degree Celsius), while ammonia-oxidizing Crenarchaeota (HWCGIII) has dominated in mat in the middle (65 degree Celsius) and lower streams (57 degree Celsius). 16S rRNA gene of nitrite-oxidizing bacteria (*Nitrospira* sp.) was also identified from mat in the lower stream. Chemical composition of the hot stream water has changed from upper to lower stream, in concert with the microbial community shift. From the upper to lower stream, ammonium content in the stream water progressively decreased (250 to 180 micro molar), while nitrate content progressive increased (7 to 40 micro molar). This suggests dissimilatory ammonia oxidation mainly by thermophilic Crenarchaeota and dissimilatory nitrite oxidation by *Nitrospira* sp. We will further discuss microbial community structure, energy and carbon fluxes of the subsurface geothermal ecosystem.