

Temperature dependent shift of microbial communities associated with muddy sediments in terrestrial hot springs

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Abstract

Terrestrial hot spring is one of the most distinct features on Earth's surface that witnesses the interaction of deeply sourced geological processes with surface hydrosphere and atmosphere. Such an interaction often creates steep temperature, chemical and redox gradients within which microorganisms proliferate in accordance with their physiological capabilities. Investigation on how microbial communities respond to the dynamics of physio-chemical properties of hot fluids would provide invaluable constraints on the delineation of the extent of habitable niches and on the factors that regulate the functional expression of microbial populations.

Of all environmental parameters, temperature fluctuation perhaps poses the most stringent effect on microbial activity in hot springs since episodic infiltration of meteoric water would substantially lower in situ temperatures (and the abundances of solutes). To mimic how thermophilic and hyperthermophilic populations respond to such short-term temperature fluctuation, we carried out a series of microcosm experiments incubated at temperatures ranging from 40 to 80°C. Sediments collected from the Kuantzeling hot spring were used as inoculums because (1) these sediments originating from marine environments provided excess organic carbon as electron donor; (2) aqueous chemistry based on previous analysis suggests that hydrothermal imprint over clay dehydration may account for the source mechanisms, rendering the reconstruction of fluid mixing from various temperature regimes possible.

Initial inspection of bulk initial aqueous chemistry suggested that sulfate and nitrate are the most plausible substrates for the terminal electron accepting processes. Therefore, artificial groundwater with 1 mM sulfate was amended with sediment slurry at a ratio of 1:1 in order to investigate whether microbial sulfate reduction dominated over the other microbial processes. Analysis of sulfate abundances yielded a sigmoid depletion of sulfate over a period of one month, whereas nitrate abundances remained unchanged. Maximum sulfate consumption rate varied from 6.6 $\mu\text{M/hr}$ at 40°C, to ~ 2.5 $\mu\text{M/hr}$ at 50°C and 60°C, and to 7.1 $\mu\text{M/hr}$ at 70°C and 12.6 $\mu\text{M/hr}$ at 80°C. Such a range of sulfate reduction rate is consistent with or greater than those

reported for similar experiment conducted on shallow hydrothermal marine sediments (ex. $\sim 3 \mu\text{M/hr}$ in Vulcano, Italy), suggesting that sulfate reducing activity in terrestrial environments was not affected by the relatively limited supply of sulfate and dissolved organic carbon when compare marine environments.

Polymerase chain reaction (PCR) amplification of 16S rRNA genes indicated a substantial reduction of bacterial biomass as the temperature increased from 60°C to 80°C. Archaeal populations were much smaller than bacterial populations given that second PCR amplification was required to detect archaeal 16S rRNA genes. Cloning and sequencing analyses yielded a significant decrease of bacterial diversity as the temperature was raised from 60°C to 70°C (13 vs. 6 phylotypes). The most dominant microorganisms at 60°C were affiliated with *Thermodesulfovibrio* spp. (37.1%) and *Thermoacetogenium* spp. (25.8%), whereas *Thermoacetogenium*- (67.9%) and *Thermoanaerobacter*- (12.5%) related microorganisms dominated over the others at 70°C. Archaeal populations were consistently composed of *Archaeoglobus* spp. no matters what temperature was used. The shift of dominant phylotypes suggested that microbial sulfate reduction was catalyzed by various groups of microorganisms at different temperatures. *Firmicutes*-related sulfate reducers outcompeted over *Nitrospira*-related sulfate reducers as the temperature increased.

Overall, microbial sulfate reduction was the dominant terminal electron accepting metabolism for organic mineralization in the Kuantzeling hot spring. The enhancement of sulfate reducing activity at higher temperatures seemed to be decoupled from growth of microbial population. While temperature variation results in a substantial change of community structure, partition of respiratory energy into biosynthesis and maintenance also warrants further investigation.