Microbial production and metabolic potential in the geothermal hot spring pool of Nakabusa, Japan

Hiroyuki Kimura[1]; Kousuke Mori[1]

[1] Institute of Geoscience, Shizuoka University

http://www.ipc.shizuoka.ac.jp/~shkimur/index.html

This study investigated the growth rate and metabolic potentials of hyperthermophilic prokaryotes in an 85^{o}C geothermal hot spring pool of Nakabusa, Japan. *In situ* cultivation using culture chambers made of stainless steel tubes was performed in order to measure the growth rate of hyperthermophilic prokaryotes. Samples of geothermal groundwater were directly collected from spring vent of the geothermal pool, and anaerobically dispensed into the cultivation chambers. The chambers were incubated in the geothermal pool, and collected at appropriate intervals. Microbial cell numbers in the incubated groundwater were directly counted with an acridine orange direct-count technique. The growth rate was estimated to be 0.43 to 0.66 day^{-1}, and which was equivalent to that of planktonic bacteria in ocean. To estimate biomass production in the geothermal pool, cellular carbon contents of hyperthermophiles were determined by using a CHN analyzer. Based on the cellular carbon contents and the growth rate, biomass production of the hyperthermophilic prokaryotes was estimated to be 4.8 to 7.4 ug of carbon per liter per hour. The microbial production in the geothermal pool suggested 19 to 117 times of that of planktonic bacteria in ocean.

16S rRNA genes of the hyperthermophilic prokaryotes in the geothermal pool were amplified by PCR with *Bacteria*- and *Archaea*-specific primer sets, and clone libraries were constructed separately. The bacterial 16S rRNA gene clones were divided into 5 operated taxonomic units (OTUs). These OTUs were closely related to the genera *Hydrogenobacter*, *Geothermobacterium*, *Sulfurihydrogenibium*, *Fervidobacterium* and *Thiomonas*. Almost bacterial clones (96% of total 49 clones) were placed beside phylogenetic clusters of thermophilic hydrogen-oxidizing bacteria. The archaeal OTUs branched into 3 phylogenetic clusters related to the genera *Desulfurococcus*, *Ignisphaera* and *Thermofilum*. All archaeal clones (40 clones) belonged to phylogenetic clusters of hyperthermophilic heterotrophs. Furthermore, bacterial and archaeal 16S rRNA genes in bulk DNA extracted from the geothermal pool were quantified by quantitative fluorescent PCR analysis. The percentage of the archaeal rRNA gene corresponded to approximately 10% of the amount of prokaryotic universal rRNA gene, which simultaneously indicated that the bacterial rRNA gene content was 90% of the prokaryotic universal rRNA gene. The quantitative PCR analysis showed that bacteria were numerically superior to archaea in the microbial community. Thus, hydrogen-oxidizing bacteria were dominant in the geothermal pool, and H_2 may play an important role as an electron donor.

Production potential of H_2 was studied by enrichment cultures of hyperthermophilic prokaryotes. The geothermal groundwater was anaerobically dispensed into sterile bottles sealed with sterile butyl rubber stoppers and aluminum crimps. Those samples were supplemented with glucose, peptone and yeast extract as substrates, and incubated at 85^{o}C. The concentrations of H_2 in the headspace were measured at appropriate intervals by using a gas chromatograph. H_2 production was observed in the geothermal water samples. Additionally, 16S rRNA gene analysis suggested the presence of hyperthermophilic bacteria belonged to genus *Fervidobacterium*, which are known as fermenting bacteria to produce H_2 and CO_2 in final phase of fermentation. The results suggested that the fermenting bacteria may supply H_2 to the hydrogen-oxidizing bacteria in the geothermal pool.