

# Microbes in million years aged geothermal aquifer associated with the deep subsurface of the Great Artesian Basin, Australia

# Hiroyuki Kimura[1]; Maki Sugihara[2]; Hiroyuki Yamamoto[3]; Kenji Kato[4]; Satoshi Hanada[5]

[1] Shizuoka Univ., Fac. Sci.; [2] AIST; [3] Marine Ecosystem, JAMSTEC; [4] Institute of Geoscience, Shizuoka University; [5] Natl. Inst. of Adv. Ind. Sci. & Tech. (AIST)

The Great Artesian Basin (GAB) is the largest artesian groundwater basin in the world, and it covers more than one-fifth of Australian continent. The GAB consists of alternating layers of water-bearing permeable sandstone and no water-bearing impermeable shale. These layers of GAB were formed between 100 and 250 million years ago (Habermehl 1980). Groundwater in the aquifer is mainly recharged by rainfall infiltrating into outcrops in the north-east margin of GAB. The water flows down through the sandstone outcrops, and then the groundwater is naturally reserved in the deep aquifer of the GAB. The flow of the groundwater is very slow at a rate from 1 to 5 m/year. Age of the groundwater is calculated within the range of  $10^5$  to  $10^6$  years old dated by isotopic ratios Kr and Cl (Collon et al., 2000; Lehmann et al., 2003). The groundwater in the GAB is warmed up by non-volcanic geothermal heat, and shows extremely high water pressure. Therefore, a vast amount of geothermal groundwater gushes out whenever a well is drilled to depth of the deep aquifer. There are a large number of free-flowing artesian wells in the GAB. The depth of these wells ranges from a few hundred meters to over 1000 m, and the temperatures (between 30C and 100C) depend on the bore depth.

To investigate subsurface microbes existed in the geothermal groundwater of the GAB, the hot groundwater gushing out from the deep aquifer was sampled and analyzed. Archaeal and bacterial small-subunit rRNA genes (rDNAs) were amplified by PCR, and clone libraries were separately constructed. The archaeal 16S rDNA clones were divided into 9 operational taxonomic units (OTUs) by restriction fragment length polymorphism. These OTUs were closely related to methanogenic *Methanospirillum* and *Methanosaeta*, heterotrophic *Thermoplasma*, or miscellaneous Crenarchaeotic group. More than a half of the archaeal clones (59% of total 59 clones) were placed beside clusters of the methanogens. Within the methanogen-related clones, the majority (83%) was closely related to a cluster of hydrogen-utilizing methanogens (genus *Methanospirillum*). In the bacterial OTUs branched into 7 phylogenetic clusters, relating to hydrogen-oxidizing thermophiles (genera *Hydrogenobacter* and *Hydrogenophilus*), a sulfate-reducing thermophile (genus *Thermodesulfobivrio*), chemoheterotrophic bacteria (genera *Thermus* and *Aquaspirillum*), or the candidate division OP10. Clones closely related to the thermophilic hydrogen-oxidizers, i.e., genera *Hydrogenobacter* and *Hydrogenophilus*, were dominant in the bacterial clone library (37% of total 59 clones).

The deep geothermal aquifer in the GAB has multilayered structure formed by mainly sandstone-layers and impermeable shale-layers of Triassic and Jurassic and Cretaceous age. It is likely that some organic matters are contained in sedimentary layers, and hydrogen is constantly produced by thermocatalysis of the organic matters in geothermal sedimentary rocks. Our analysis based on 16S rDNA sequence suggested that hydrogen-utilizing methanogens and hydrogen-oxidizing bacteria predominantly inhabit the geothermal aquifer in the GAB. These microbes able to hydrogen as a sole energy source probably play an important role in the deep geothermal aquifer as primary producers.

The microbial ecosystem in the deep aquifer was supported by hydrogen produced by abiotic thermocatalysis of organic matters in sedimentary layers. Since the biotic compounds mainly originated in the ancient photosynthesis, it can be said that the subsurface microbial ecosystem in the GAB is independent from the current photosynthetic ecosystem in surface environment of the earth. Therefore, the subsurface microbes in the GAB would be survive a long-time geological event that seems to be undesirable for all terrestrial life such as Snowball Earth and the impact of a huge meteorite.

Habermehl (1980) BMR J. Aust. Geol. Geophy. 5:9-38.

Collon et al. (2000) Earth Planet. Sci. Lett. 182:103-113.