BIOGENIC SILICA DEPOSITION IN GEOTHERMAL HOT WATER

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SUMMARY – Current observations of geothermal and hydrothermal deposits have suggested that a variety of thermophilic or hyperthermophilic microorganisms living under such extreme conditions are likely to be associated with the rapid formation of siliceous deposits from hot waters. Culture-independent molecular ecological studies revealed that the extremely thermophilic microbial population was consistently present in silica scales formed in the aging tank at the Japanese geothermal power plant and the genera *Thermus* and *Hydrogenobacter* were predominant microbial components in the siliceous deposits. Culture-dependent in vitro analyses using the *Thermus* cells indicated that the cell growth caused the rapid precipitation of supersaturated amorphous silica and concomitantly produced a specific cell envelope protein, we called 'Sip (silica induced protein)'. Our study implicates that microorganisms may utilize the dissolved supersaturated amorphous silica for the maintenance of position and survival in limited niches in geothermal hot water environments.

1. INTRODUCTION

Since the hot water fluid originated from deep underground is often highly saturated with respect to amorphous silica, siliceous deposits are formed in geothermal and hydrothermal environments. On the basis of the research at Yellowstone National Park, Walter *et al.* (1972) first suggested that microorganisms might affect the formation of the siliceous deposits such as sinter and geyserite, and some of these textures are similar to Precambrian stromatrites. The formation of siliceous deposits from geothermal waters is strongly affected by the cooling and evaporation, with numerous microbial fabrics coated with amorphous silica having been observed in hot springs and deep-sea hydrothermal environments (eg. Konhauser and Ferris 1996, Jones and Renaut 1996, Inagaki *et al.,* 1997, 2001, Jones *et al.,* 2001, Al-Hanbali *et al.,* 2001). These observations infer that viable populations of microorganisms are present in hot water fluids even at temperatures greater than boiling and may affect the rapid deposition of dissolved chemical components.

The study presented here addresses the issue of a microbial community associated with the siliceous deposits formed at Japanese geothermal power plant. The formation of silica scales in pipelines of geothermal power plants presents serious economic problems, related to energy losses and increased costs of cleaning and maintenances. In order to control the formation of silica scale, geothermal water is temporarily stored in aging tanks, and siliceous deposits in the tank are then removed. However, in our research, since microorganisms may affect the aggregation of silica, the formation rate is higher than expected, there are no measures to control the formation of silica scale. Culture-independent molecular analyses and culture-dependent in vitro analyses demonstrated that *Thermus* cells, one of the predominant indigenous microbial components in the siliceous deposits, were strongly associated with the rapid deposition of supersaturated amorphous silica. We discuss here the role and significance of dissolved amorphous silica for the thermophilic microorganisms and the mechanisms for biogenic silica deposition in geothermal hot water environments.

2. MATERIALS AND METHODS

2.1 Silica scale

Silica scale used in this study were formed on thin copper plates immersed in the aging tank (85±2°C, pH 7.2) for 2 months at the Otake geothermal power plant, Kyushu, Japan (Inagaki *et al.,* 1997) (Fig. 1). The geothermal hot water was injected into the aging tank at a flow rate of 350 tons per hour. Silicic acid in the geothermal hot water at the aging tank was approximately 710 ppm. The silica scale was mainly composed of amorphous silica (90.31 wt%), containing trace amounts of Al and Fe (XRD data, Inagaki *et al.,* 1997). The silica scales on the plates were frozen immediately in liquid nitrogen, and stored at –20°C prior to use in laboratory.

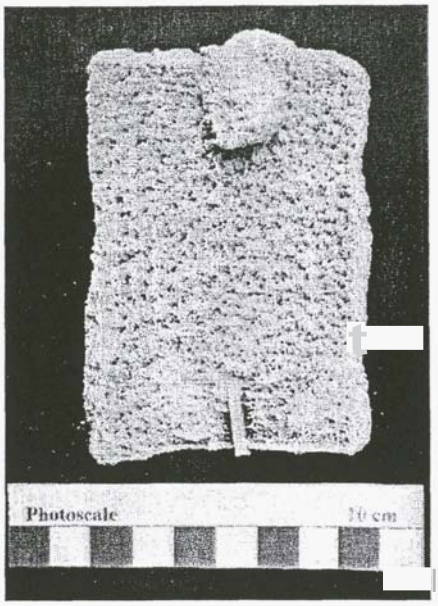

Figure 1 Silica scale formed on a cupper plate. The plate was incubated for 40 days in hot water (85±2°C, pH 7.2) at the Otake geothermal power plant, Kyushu, Japan.

2.2 Microscopy

Silica scale was freeze-dried overnight, and then observed by electron-probe (EPMA)(JEOL JXA-733). The cells were negatively stained and observed by transmission electron microscopy (TEM)(JEM 2000EX).

2.3 Extraction of DNA and sequencing of bacterial 16S rDNA

Bulk endolithic DNA was extracted from the frozen silica scales by the lysozyme and freeze-thaw method (Inagaki et al., 1997). The extracted DNA was concentrated by ethanol precipitation, and then purified with a Qiagen- column (Qiagen Inc.) according to the manufacturer's instructions.

Partial bacterial 16S rDNA was amplified by PCR, using universal primers U1101 (5'-AACGAGCGMRACCC-3') and U1392 (5'-GACGGGCGGTGTGTRC-3') as previously described (Inagaki *et al.,* 1997). The rDNA fragment was cloned in vector pUC119, and then sequenced from both strands using a DNA sequencer (DSQ-500, Shimadzu Co.).

2.4 Phylogenetic analysis

The determined partial rDNA sequences were compared with DNA sequences deposited in GenBank/EMBL/DDBJ databases by the BLAST network service. The phylogenetic trees were constructed by the neighbour-joining and UPGMA methods based on 275 bp of homologous positions.

2.5 Isolation of *Thermus* spp. from silica scale

0.5 g of frozen silica scale was directly put into the TM broth medium containing 0.4 wt% tryptone, 0.2 wt% yeast extract, 0.1 wt% NaCl, and Castenholz basal salt (pH=7.2), and then incubated for a few days at 80°C. The enrichment culture was inoculated to the solid TM medium containing 3.0% agar and incubated at 70°C. This step for single isolation was preformed several times, and the strain TMY was obtained (=JCM10668).

2.6 In vitro analysis of biogenic siliceous deposition

Thermus sp. strain TMY, *T. thermophilus* ATCC-27634, *T. flavus* ATCC-33923, and *T. aquaticus* ATCC-25104 were cultivated in the TM broth medium containing 0-650 ppm (as SiO_2) sodium meta-silicic acid (Wako Co.). The concentration of silicic acid in culture medium was measured by the inductively coupled plasma emission spectrometer (IRIS, Thermo Jarrel Ash Co.) or by the molybdenum-yellow method (Inagaki *et al.,* 1998). Before the measurement of silicic acid, the pH in culture medium was adjusted to 3.0 to prevent the further polymerisation of silicic acid.

2.7 Profiling the cell envelope proteins

Thermus cells grown in the TM broth medium containing supersaturated amorphous silica (500-600 ppm at 75°C) were harvested at the late-exponentially growth phase, and washed twice with 50 mM Tirs-HCl buffer (pH 8.0). Cells were broken by a brief sonication for 10 sec (Ultrasonic Disruptor UD201, Tomy), and cell envelope fractions were recovered from the supernatant by the ultra-centrifugation at 279 000*g* for 1 h (Optima™ TLX Ultracentrifuge, Beckman). The pellet was resuspended with 50 mM Tirs-HCl buffer (pH 8.0) containing 5% (v/v) SDS. The composition of cell envelope proteins under the various concentrations of silicic acid was monitored by 10% (w/v) SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The slab gel was stained with Coomassie brilliant blue R250 to visualize proteins.

3. RESULTS AND DISCUSSION

3.1 Thermophilic microbial community in silica scale

EPMA observation revealed that the silica scale was composed of an aggregate of numerous microbial fabrics and inorganic spherical silica grains (Fig. 2). DNA was constantly and stably extracted from the silica scale with increasing the amount of silica scale on the copper plate for 2 months (average 8 μg/ 1 wetg of silica scale

(Inagaki *et al.*, 1997). Based on these results, the thermophilic microorganisms were consistently present in the silica scale during the formation process and likely to be associated with the rapid deposition of amorphous silica from geothermal hot water. Although the examined length of rDNA fiagments were very short, phylogenetic analyses of PCR-amplified bacterial 16S rDNA sequences indicated that the bacterial community inside the silica scale was clearly composed of two genera: ***Thermus*** and ***Hydrogenobacter*** (Inagaki ***et al.,*** 1997, 2003). The genus ***Thermus*** is a heterotrophic, extreme thermophilic bacterium phylogenetically located within the ***Thermus-Deinococcus*** group, while the genus ***Hydrogenobacter*** is normally an autotrophic, hydrogen-oxidizing bacterium within the order ***Aquificales***. The genus ***Thermus*** and hydrogen oxidizing bacteria within the order ***Aquificales*** have often been isolated from terrestrial hot springs all over the world. In Yellowstone National Park, one of the most active geothermal areas on Earth, we found the ***Thermus*** community on siliceous sinter at the Steep Cone hot spring (Inagaki ***et al.,*** 2001). Recently, bacterial communities occurring with near-boiling silica sinter or geyserite in Yellowstone National Park were reported, which were composed of members within ***Aquificales*** (Blank ***et al.,*** 2002). The microbial ecosystem composed of heterotrophs and autotrophs seems to be a common feature occurring on the siliceous deposits at high temperature area (>70°C) in neutral hot springs.

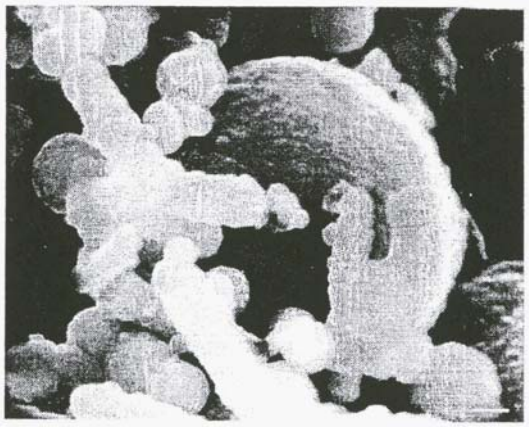

Figure 2 EPMA micrograph of silica scale formed on the plate in the geothermal hot water. The scale was composed of silicified microbial fabrics and spherical particles of amorphous silica. Bar=1 μm.

3.2 In vitro biogenic silica deposition

We isolated an extremely thermophilic heterotrophic bacterium strain TMY, from the silica scale formed in the aging tank at the Otake geothermal power plant. An almost full length of 16S rDNA sequence of thPis isolate was determined (Database Accession No. AB020888). The similarity analysis indicated that the strain **TMY** was closely similar with the sequence of ***Thermus*** sp. strain HS1A.1 isolated from the Hanmer hot spring in southern New Zealand and *T. thermophilus* HB8 isolated from a Japanese hot spring, indicating that the isolates were likely to be one of the species of *T. thermophilus*. This strain was deposited at the Japan Collection of Microorganisms (=JCM10668).

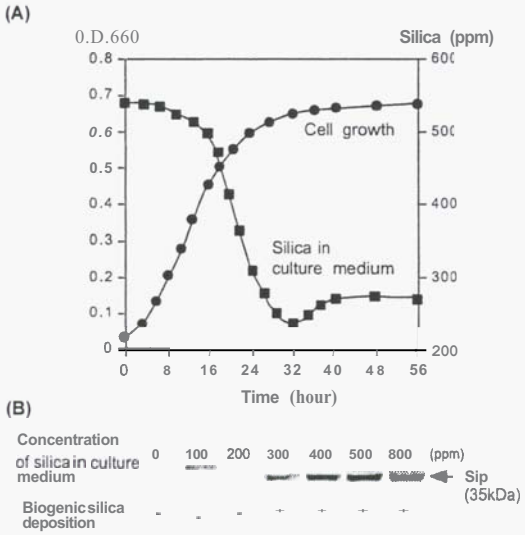

Figure 3 Biogenic in vitro silica deposition by an extreme thermophilic bacterium Thermus **sp.** Strain TMY isolated from silica scale. (A) Profiles of cell growth and concentration of silica in culture medium supernatant. Cultivation was performed at 75°C. (B) Induction of cell envelop protein by various concentrations of amorphous silica. SDS-PAGE was performed using 10% (w/v) polyacrylamide gel. The expression of a protein of 35kDa, Sip, was induced by the presence of supersaturated polymeric silica. Expression of Sip was concomitant with in vitro biogenic silica deposition.

Using the isolated strain TMY and other ***Thermus*** species, we performed in vitro experiments for biogenic deposition of amorphous silica. As shown in Fig. 3, supersaturated silica was effectively precipitated in vitro during the exponential growth phase of ***Thermus*** strains (Inagaki ***et al.,*** 1998, 2003). Since bacterially mediated silica deposition hardly occurred at under-saturated concentration of silicic acid, the cells might precipitate silica only in the case of supersaturated polymeric silica. In addition, the profiling of cell envelop proteins under various concentrations of silica in culture revealed that one insoluble protein was specifically induced by the presence of supersaturated polymeric silica. We called this protein 'Sip (silica induced protein)'(Inagaki ***et al.,*** 2003). The molecular mass of Sip was approximately 35kDa, and we found the expression of Sip in every ***Thermus*** cultures. The expression of Sip was observed in correspondence with biogenic silica deposition.

These results implied that *Thermus* cells might possess molecular mechanisms associated with extracellular biogenic silica precipitation.

3.2 Ecological significance of silica for Extremphiles and industrial applications

Microbial cell surfaces and extracellular polymers may serve as nucleation sites for secondary mineral phases, through which mineral precipitation reactions can occur (Urmtia and Beveridge 1994, Konhauser and Ferris 1996). We find that thermophilic microorganisms living in geothermal hot water are strongly associated with the rapid formation of siliceous deposits. However, it is not well understood why these thermophilic microorganisms use amorphous silica in natural geothermal environments. Silica is not a necessary component for microbial cell growth because it does not act as an electron acceptor or donor. On one hand, high concentration of silica sometimes shows little toxicity on cell growth. Since the habitats for extreme thermophiles and hyperthermophiles in geothermal environments are limited to temperature ranges from 70°C to boiling point, such microorganisms must attach to deposits in order to survive and maintain their position in appropriate niches. As described above, *Thermus* strains promote the formation of siliceous deposits and produce specific cell envelope protein Sip in the presence of supersaturated polymeric silica. Sip may participate in interactions between cell surface and silica deposits in natural geothermal environments.

Study of the relationship between siliceous deposition and microorganisms will be significant fiom the industrial point of view. Population and physiological characteristics of predominant microbial communities associated with silica scales will shed light on how to control bio-mediated silica scale formation in geothermal power plants (e.g. pH, temperature, salinity, dissolved oxygen control). Furthermore, the rapid formation of biogenic silica may be utilized to remove dissolved toxic metals such as Al, As, Hg Fe, Pb, Se, etc. from polluted waters. Although the study of the mechanisms of microbial silica deposition has just begun, it probably has great industrial and ecological potentials.

4. ACKNOWLEDGEMENTS

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5. REFERENCES

Al-Hanbali, H.S., Sowerby, S.J., and Holm, N.G. (2001). Biogenicity of silicified microbes from a hydrothermal system: relevance to the search for evidence of life on earth and other planets. ***Earth Planet. Sci. Lett.,*** 191, 213-218.

Blank, C.E., Cady, S.L., and Pace, N.R. (2002). Microbial composition of near-boiling silica-depositing thermal springs throughout Yellowstone National Park. ***Appl. Environ. Microbiol.,*** 68,5123-5135.

Inagaki, F., Hayashi, S., Doi, K., Motomura, Y., Izawa, E., and Ogata, S. (1997). Microbial participation in the formation of siliceous deposits from geothermal water and analysis of the extremely thermophilic bacterial community. *FEMS* ***Microbiol. Ecol.,*** 24, 41-48.

Inagaki, F., Yokoyama, T., Doi, K., Izawa, E., and Ogata, S. (1998). Bio-deposition of amorphous silica by an extremely thermophilic bacterium, ***Thermus*** spp. ***Biosci. Biotechnol. Biochem.,*** 62, 1271-1272.

Inagaki, F., Motomura, Y., Doi, K., Taguchi, S., Izawa, E., Lowe, D.R., and Ogata, S. (2001). Silicified microbial community at Steep Cone hot spring, Yellowstone National Park. ***Microb. Environ.,*** 16, 125-130.

Inagaki, F., Motomura, Y., and Ogata, S. (2003). Microbial silica deposition in geothermal hot waters. ***Appl. Microbiol. Biotechnol.,*** 60,605-611.

Jones, B., and Renaut, R.W. (1996). Influence of thermophilic bacteria on calcite and silica precipitation in hot springs with water temperature above 90°C: evidence fiom Kenya and New Zealand. ***Can.*** *J.* ***Earth Sci.,*** 33, 72-83.

Jones, B., Renaut, R.W., and Rosen, M.R. (2001). Biogenicity of gold- and silver-bearing siliceous sinters forming in hot (75°C) anaerobic spring-waters of Champagne Pool, Waiotapu, North Island, New Zealand. *J.* ***Geol. Soc.*** (London), 158, 895-911.

Konhauser, K.O., and Ferris, F.G. (1996). Diversity of iron and silica precipitation by microbial mats in hydrothermal waters, Iceland: Implications for Precambrian iron formations. ***Geology*** 24,323-326.

Urrutia, M.M., and Beveridge, T.J. (1994). Formation of fine-grained metal and silicate precipitations on a bacterial surface ***(Bacillus subtilis). Chem. Geol.*** 116,261-280.

Walter, M.R., Bauld, J., and Brock, T.D. (1976). Siliceous algal and bacterial stromatolites in hot spring and geyser effluents of Yellowstone National Park. ***Science,*** 178,402-405.