Preliminary Communication

Bio-deposition of Amorphous Silica by an Extremely Thermophilic Bacterium, Thermus spp.

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The bio-deposition of amorphous silica, which occurred in vitro by exposure to the extremely thermophilic bacterium Thermus spp., began from the latter part of the exponential phase of growth of the bacteria. The concentration with which the deposition occurred exceeded the solubility of amorphous silica of neutral pH at the temperature 60–85°C. Our observations suggest that Thermus spp. promotes the formation of siliceous minerals in a geothermal environment.

Key words: silica; Thermus spp.; bio-deposition; geothermal water

Siliceous deposits forming in geothermal water can cause serious economic problems related to energy loss and maintenance at geothermal power plants throughout the world. When we investigated the biological aspects of properties of siliceous deposits we found numerous bacteria-like structures in the deposits. We then extracted DNA from the deposits and assessed the correlation between the amount of DNA and the rate of formation of silica. The microbial population was analyzed using 16S rDNA, and was composed of 3 clusters of thermophiles: the genus Hydrogenobacter, the genus Thermus, and anaerobic Gram-positive bacteria. Our work suggested that these extremely thermophilic bacteria participated in forming the siliceous deposits.

We now report the bio-deposition of silica in vitro, the first documentation that microorganisms can affect silica deposition at high temperatures.

An extremely thermophilic Thermus sp., strain TMY, was readily isolated from siliceous deposits that formed at 80–85°C in geothermal waters of a geothermal electric power plant, Kyushu, Japan. This new strain can grow at 50–88°C and the optimum temperature for growth was approximately 75°C. Genus Thermus was the dominant species of bacteria in the siliceous deposits. Thermus sp. strain TMY, T. thermophilus HB8 (ATCC-27634), T. flavus AT-62 (ATCC-33923), and T. aquaticus YT-1 (ATCC-25104) were cultivated in TM liquid medium containing silicic acid at 75°C, with continuous shaking. A stock solution of silicic acid was prepared by dissolving sodium metasilicate in 0.1 N NaOH. The pH in the medium was adjusted to 7.2 with HCl followed immediately by autoclaving. The strains were then inoculated into the medium without cooling it down. Figure 1 shows X-ray diffraction patterns (XRD) of the deposits. The broad band indicates that all the deposits were composed of amorphous silica like siliceous deposits that formed in the presence of geothermal water.

Figure 2 shows changes in pH and in concentrations of silicic acid during growth of strain TMY at 75°C. Portions of the supernatants of these cultures were added to 0.1 N HCl to prevent the polymerization of silicic acid, then the total concentration of silicic acid was measured using an inductively coupled plasma emission spectrometer (IRIS, Thermo Jarrell Ash Co. U.S.A.). From 24 to 32 hours after inoculation, growth of the bacteria changed from exponential to stationary phase and the pH increased approximately from 7.2 to 9.2 during growth. Silicic acid concentrations in the medium slightly decreased during the early stage of the exponential growth phase, however, from the latter stage of the exponential growth phase, the silicic acid concentration decreased abruptly, indicating initiation of a drastic deposition of silica. After about 40 hours, the total concentration of silicic acid remained constant at about 280 ppm (SiO₂). This concentration was less than the solubility of pure amorphous silica at 75°C, indicating that the bio-deposition formed was stable and of lower solubility. The same phenomena were observed with other

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Thermus strains, T. thermophilus, T. flavus, and T. aquaticus. We also measured the concentration of monosilicic acid, based on the formation of yellow molybdosilicic acid. Since the monosilicic acid concentration remained constant at about 280 ppm during incubation at 75°C (data not shown), we conclude that the bio-deposition was likely to be caused by aggregation of polysilicic acid particles, formed by polymerization of supersaturated monosilicic acid.

Figure 3 shows the total concentration of silicic acid in the medium after 48 h of incubation at 60, 70, and 80°C using Thermus spp.. The bio-deposition began from the concentration of about 200, 300, and 400 ppm at 60, 70, and 80°C, respectively. In the control incubation excluding all bacteria, total concentrations of silicic acid exceeded 400 ppm (SiO₂) and only about 10–20% of total silicic acid was deposited inorganically during 48 hours. Approximately 50–60% of the total silicic acid was deposited during growth of the bacteria. At 80°C, the bio-deposition did not occur before 400 ppm, otherwise the final constant concentration of silicic acid was about 300 ppm. These constant concentrations of silicic acid after the bio-deposition correspond to the solubility of amorphous silica of neutral pH at each temperature. It can be reasonably concluded that bacteria precipitate all of the polysilicic acids over the solubility of amorphous silica of neutral pH during their growth.

Our observations of the deposition of silica in vitro suggest that bacteria contribute to increasing the aggregation of polysilicic acid in hot water, even in natural geothermal environments. Proteins or lipids on the cell surface produced at latter stage of the exponential growth phase may relate to the deposition of silica. We are planning to examine some dominant thermophilic bacteria other than Thermus spp. in other siliceous deposit at the geothermal field. We hope our study will contribute to methods which will retard growth of silicic deposits at geothermal electric power plants as well as to clarification of mechanisms of the bio-deposition of amorphous silica.

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References