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# Bacteria as nucleation sites for authigenic minerals

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**Abstract**

**Microbial mats are densely entangled with their mineral substrates and organized in response to the sediments by the products of biotransfer. The adsorption of dissolved constituents from the aqueous environment is considered to contribute significantly to the mineral formation process. Microbial mats from Iceland, Korea and Japan have been analyzed from mineralogical, biological and micromorphological viewpoints. Scanning electron microscopy equipped with energy dispersive X-ray spectroscopy and transmission electron microscopy of thin sectioned samples revealed a variety of elemental concentration in encrusted cyanobacteria. X-ray powder diffraction patterns and corresponding electron diffraction patterns indicated calcite, buserite, cristobalite, carbonate, silicates, Mn and Fe oxides. The authigenic grains exhibited a wide range of morphology, i.e., from amorphous gel-like phases to crystalline structures. Numerous microorganisms, particularly cyanobacteria such as *Anabaena* sp. (*Synechococcus* sp. and *Synechocystis* sp.), *Oscillatoria* sp. and *Nostoc* sp., are encrusted by minerals surrounding cell walls or colonial surfaces. Microbial mats play a key role in the concentration of these elements and bacterial mineralization that will ultimately become part of the sediments.**

*Key words : Si, Ca, Mn and Fe concentration, microbial mats, biomineralization, cyanobacteria, XRD, XRF, SEM-EDX, TEM.*

## Introduction

Microbial mats are, and have been in the geological past, extremely wide-spread biological systems. It can be argued that microbial mats have controlled biotransfer processes and the biogeochemical cycles during most of the history of life on the earth (Krumbein, 1979). Cyanobacteria are frequently observed as framework builders in the case of siliciclastic and carbonate stromatolites. Microbial activity is an important factor in modifying the surface of our planet and the ecosystem (S. Mann et al., 1989 ; Simkiss and Wilbur, 1989 ; Mitchell, 1992 ; Fyfe, 1993 ; Tazaki et al., 1995). An enormous amount of carbon dioxide has been fixed in carbonate deposits during the evolution of life. High-magnesium calcite are commonly found in cemented marine carbonate accumulations from calm water lagoons and microcavities in reefs (Chafetz, 1986). The role of photosynthetic cyanobacteria in the formation of calcareous microbial deposits in alkaline freshwater lakes or in hot springs has been reported by Thompson et al. (1990), Thompson and Ferris (1990), Kempe et al. (1991), Ford and Mitchell (1992) and Tazaki (1995a). The distribution and types of microorganisms preserved as cellularly intact microfossils are remarkably similar to those of modern microbial mats. But, the role of Si, Ca, Mn and Fe compounds in relation to productivity of microorganisms in microbial mats is not well understood. Little is known about the effects of pH and temperature on biomineralization. This article presents examples of Si, Ca, Mn and Fe concentration by cyanobacteria in microbial mats. Electron microscopy techniques have revealed successive stages of bacterial mineral formation of silicates, carbonates, Mn and Fe oxide compounds. Bacterial biomineralization and bacterial colony growth, microbial reactions producing these compounds in the microbial mats are discussed in this paper.

## Materials and methods

Microbial mats, green, yellow, black, white and brown in color, were collected from hot springs and river sediments where cyanobacteria were prolific in Iceland, Korea, Japan and New Zealand. The hot spring deposits and river sediments are characterized by their various color, indicating extensive biomineralization of the bacterial sheaths that constitute

**Table 1.** Biomineralization of Si, Ca, Mn and Fe spherules associated with cyanobacteria in microbial mats from hot springs and river freshwater.

| Sample | pH / Temp. (℃) | XRD | SEM- EDX, TEM |
|---|---|---|---|
| Iceland, 703-5D | 8.1 / 20.6 | calcite | Ca-spherule |
| 703-5C | 6.8 / 39.0 | amorphous | Ca-and Fe- spherules |
| 702-5A | 9.5 / 33.0 | 4 - 3.3 Å | Si-spherule |
| Korea, Sokcho | 7.7 / 30.3 | buserite | Mn-spherule |
| Japan, Kanazawa | 7.2 / 16.0 | amorphous | Fe-and Mn-spherules |
| Japan, Abashiri | 1.6 / 32.6 | cristobalite | Si-spherule |
| Japan, Nakadatsu | | amorphous | Mn-and Ca-spherules |

the predominant material in the seeps. Microbial mats in Iceland, Geyser (702-5 A) and Lysuholl (703-5 C and 703-5 D) in Snae Fellsnes Peninsula, north west of Reykjavik, were collected in the summer of 1992. Temperature of the freshwater in hot springs ranged from 12.4-89.5℃ at pH 6.3-9.6. Most of the microbial mats were found in the springs at over 30℃ under alkaline conditions (Table 1). Sokcho in Korea is one of the rare spots to produce hot springs. The black microbial mats were observed in this area at pH 7.7 and 30.3℃. Japan has many hot springs at almost all places, such as volcanic active hydrothermal areas. One of the strong acidic hot springs is located in Abashiri, Hokkaido which has produced green microbial mats at pH 1.6 and 32.6℃. River freshwater sediments have widely produced brown/black microbial mats in Kanazawa and Nakadatsu. These microbial samples are listed up in Table 1.

Phase contrast and differential interference contrast microscopes (Nikon Optiphot-2) were used for the identification of microorganisms in wet natural samples. Minerals were identified by using characteristic d-spacings from the X-ray powder diffraction (Rigaku goniometer, Cu K$\alpha$ radiation) in bulk dry powder samples. Minerals on the surface of cell wall in thin sectioned samples were identified by electron diffraction pattern of selected area.

Both microorganisms and secondary materials were observed by scanning electron microscopy (SEM) equipped with energy dispersive X-ray analyzer (EDX) and transmission electron microscopy (TEM). TEM image of encapsulated bacteria in unstained microbial mats of thin sectioned samples was obtained with a JEOL 2,000EX TEM, operating at 160 kV. SEM (JEOL JSM-T220 A) with EDX, operating at 15-20 kV, was used for the micromorphology and chemistry of the bulk samples. XRF (JEOL-JSX-3,200) was used for elemental analyses of bulk microbial mats collected from four places including samples from Waiotap in New Zealand.

**Table 2.** Chemical compositions of microbial mats by XRF, showing bacterial concentration of elements.

| Microbial mats | 1 wt% | 2 wt% | 3 wt% | 4 wt% |
|---|---|---|---|---|
| $Na_2O$ | 1.05 | - | - | 0.61 |
| MgO | - | - | - | - |
| $Al_2O_3$ | - | - | 4.22 | - |
| $SiO_2$ | 24.97 | 8.84 | 9.89 | 1.21 |
| $P_2O_5$ | - | 0.45 | 0.04 | - |
| $SO_3$ | 69.73 | 0.31 | 0.22 | - |
| $K_2O$ | 0.24 | - | 0.42 | - |
| CaO | - | 2.94 | 4.81 | 10.3 |
| $TiO_2$ | - | - | 0.19 | - |
| MnO | - | 0.93 | 76.80 | 83.72 |
| $Fe_2O_3$ | 0.30 | 86.45 | 2.63 | 0.10 |
| CuO | - | - | - | - |
| ZnO | - | - | 0.15 | 2.06 |
| $As_2O_3$ | 3.71 | - | - | 0.19 |
| $Rb_2O$ | - | - | - | - |
| SrO | - | 0.07 | 0.32 | 0.22 |
| $Y_2O_3$ | - | - | 0.31 | - |
| $ZrO_2$ | - | - | - | - |
| $MoO_3$ | - | - | - | 0.49 |
| BaO | - | - | - | 0.21 |

1: Waiotap, N.Z., 2: Kanazawa Univ., Japan,
3: Sokcho, Korea, 4: Nakadatsu, Japan.

## Results

### XRF results

XRF analyses of the bulk samples of the microbial mats collected from the hot springs in Waiotap, New Zealand (N.Z.) and Sokcho, Korea and from freshwater in Kanazawa and Nakadatsu, Japan show the presence of various elements and different ratio between them (Table 2). The microbial mats from Waiotap are rich in $SO_3$ (70%) whereas other three samples are

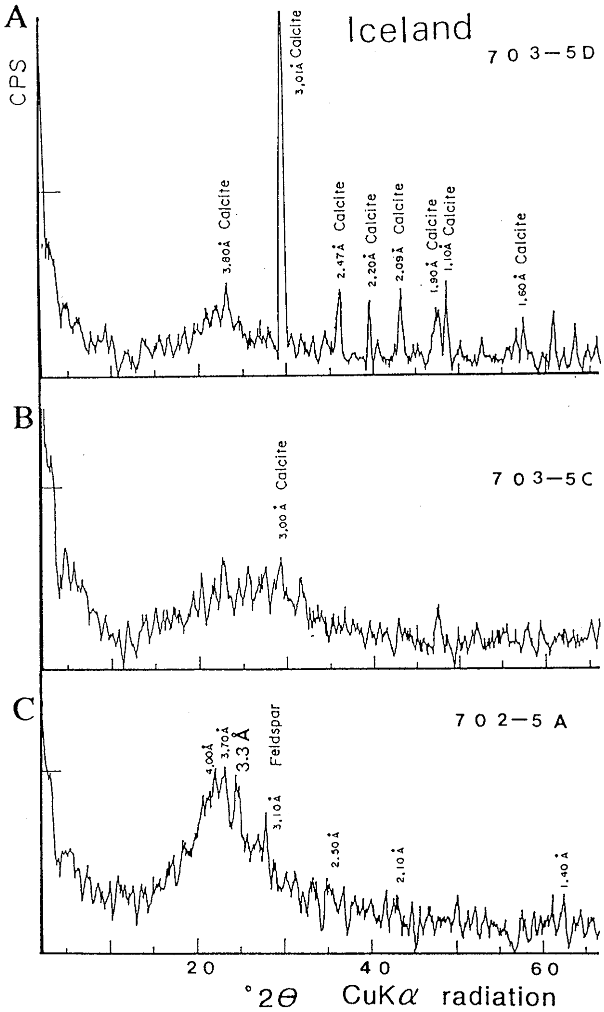



**Fig. 1.** X-ray powder diffraction patterns of bulk samples of microbial mats in hot springs from Lysuholl (703-5 D, 703-5 C) and Geyser (702-5 A), Iceland, showing calcite formation and amorphous materials.

rich in MnO and $Fe_2O_3$ (77-86%). High $SiO_2$ content (25%) in Waiotap sample is due to the presence of diatoms. Relatively high $As_2O_3$ and SrO (0.2-3.7%) are concentrated in microbial mats. Accumulation of these toxic elements in microorganisms will be discussed in a separate paper.

**XRD results**

X-ray powder diffraction of the bulk samples of the microbial mats collected from hot springs in Iceland shows the presence of well-crystallized calcite at Lysuholl (703-5 D). Amorphous materials were identified in the bulk samples collected from other hot springs (703-5 C and 702-5 A) with broad d-spacings at 3.3-4.0 Å (Fig. 1). The d-values of 3.01, 2.47, 2.20 and 2.09 Å are characteristic peaks of calcite. A high back ground between 15-30° suggests the presence of amorphous and/or organic materials. The HCl test carried out in the field at Geyser (702-5 A) indicates that the microbial mats are lack of carbonates. The

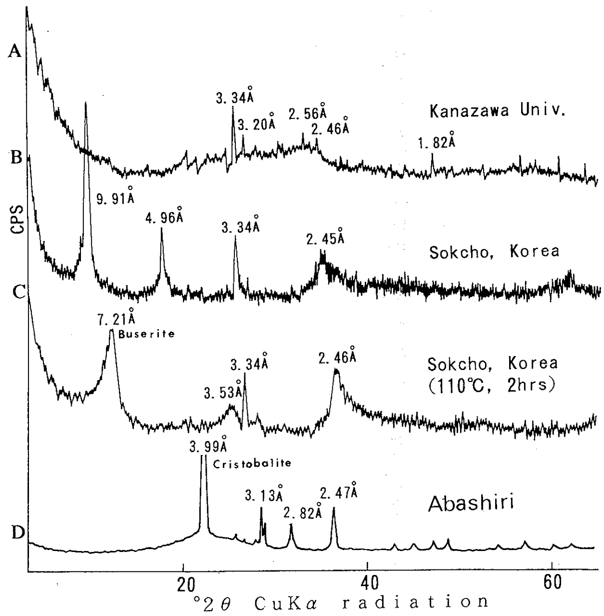



**Fig. 2.** X-ray powder diffraction patterns of bulk samples of microbial mats in river freshwater in kanazawa, in hot springs in Sokcho, Korea, and Abashiri, Hokkaido, showing amorphous materials, buserite and cristobalite formations.

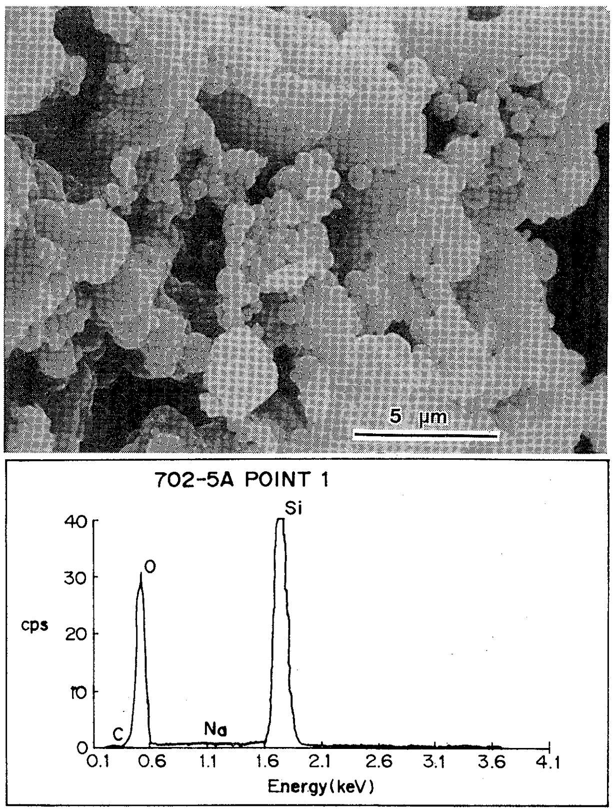



**Fig. 3.** Scanning electron micrograph and EDX of Si-concentrated spherules from Geyser, Iceland (702-5 A).

d-value of 3.3-4.0 Å of a high back ground in the sample suggests the presence of amorphous siliceous materials, whereas EDX data indicated the presence of

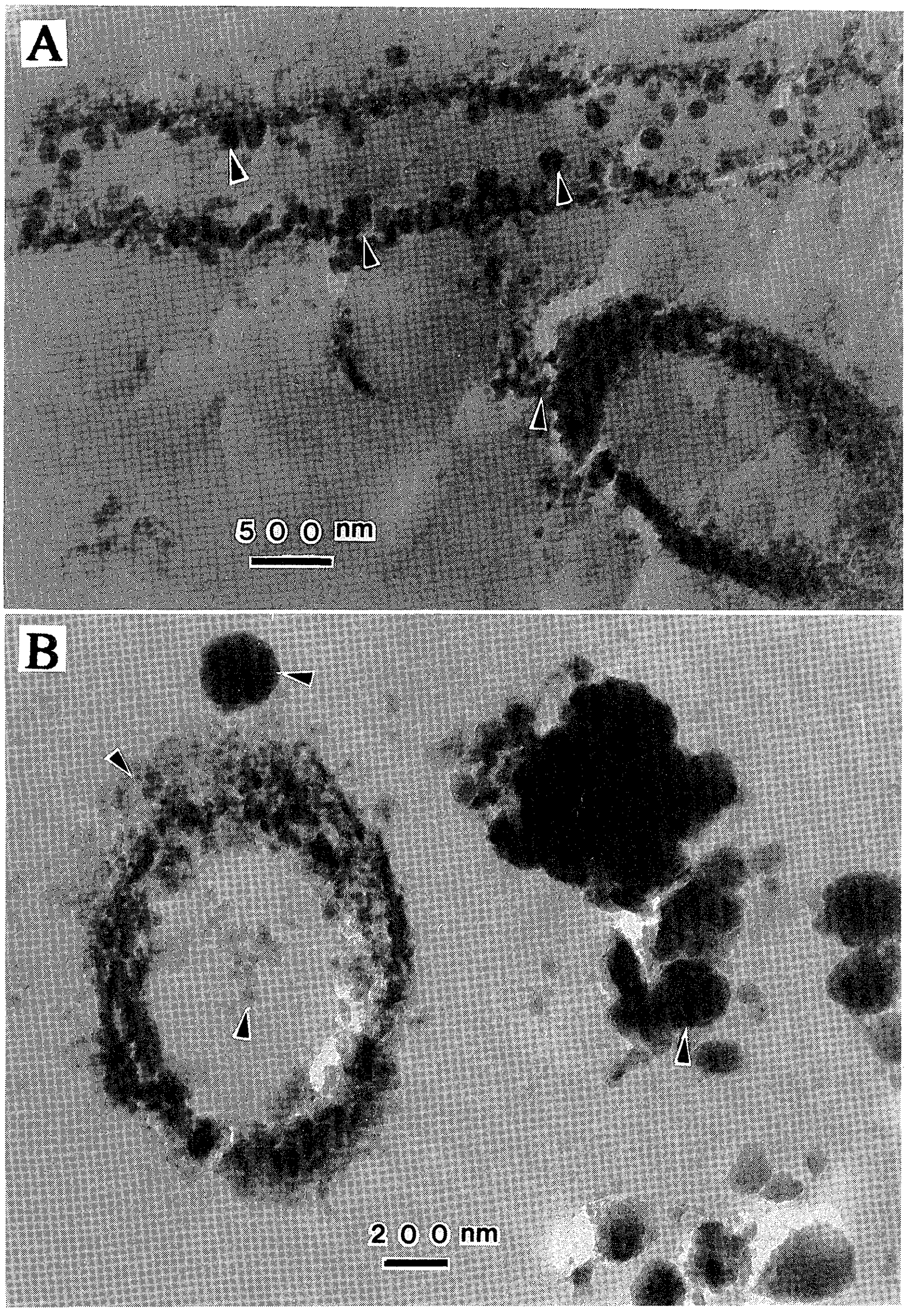
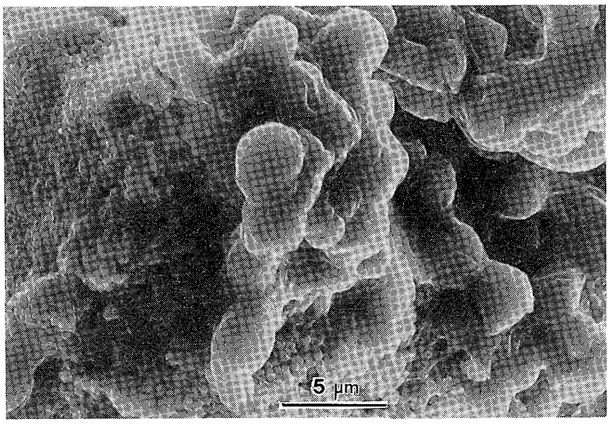



**Fig. 4.** TEM micrographs of ultra thin section of Si-concentrated spherules from Geyser (702-5 A). The mineralized cells of *Nostoc* sp. *orcynechocystis*. EDX data of the cell wall show a high silica content.

silicate compounds. Iron oxides (2.5–2.6 Å) are low crystalline materials which was identified by Fe signals through EDX analysis of the bulk samples. XRD data of the bulk microbial mats from Kanazawa show the presence of small amounts of quartz at 3.34 Å and feldspars at 3.20 Å, in addition to abundant amorphous materials (Fig. 2). The black microbial mats in Sokcho are mainly composed of buserite at 9.91 Å, which was identified by its peak-shift to 7.21 Å by heating treatment at 110℃, 2hrs. XRD data of the green microbial mats in Abashiri show the presence of pure well crystallized cristobalite (Fig. 2).

**Optical microscope observations**

Phase contrast and differential interference contrast of light photo-microscopes show an abundance of coccoidal cyanobacteria with tube-like sheath structure and fibrous cyanobacteria. Blue-green filaments are identified as cyanobacteria and many different species. The most common cyanobacteria are of *Oscillatoria* sp. found at many hot springs. *Nostoc* sp. in Lysuholl (703-5 C and 5 D), *Synechococcus* sp. and *Synechocystis* sp. are also commonly found in hot springs. Colonial *Gloeothece* sp. is found only in Lysuholl (703-5 D). Coccus and bacillus (rod) of bacterial cells form colonies. These organisms were likely a member of the bacterial genus *Synechocystis* sp. and *Synechococcus* sp., respectively. Some organisms of *Oscillatoria* sp. are encrusted with



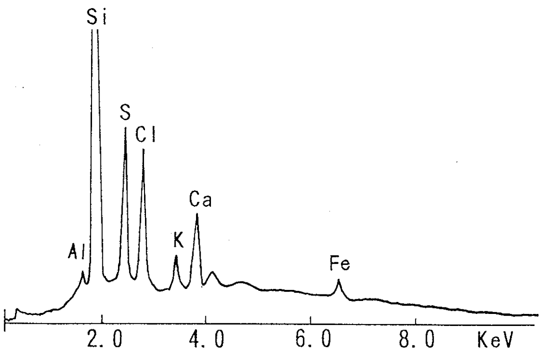




**Fig. 5.** Scanning electron micrograph and EDX of Si-concentrated spherules with traces of S, Cl and Ca from Abashiri in Hokkaido. XRD data of the green microbial mats showed presence of well crystallized crystobalite.

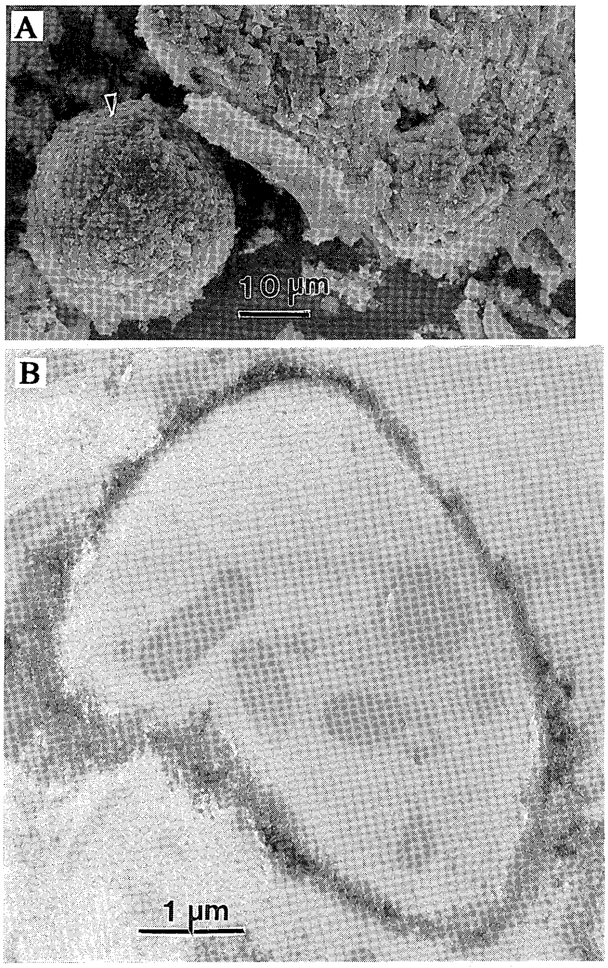



**Fig. 6.** SEM (A) and TEM ultra-thin section (B) micrographs of calcite spherules from Lysuholl (703-5 D), showing carbonate mineralization surrounding bacterial colonies. A large spherule was formed by several living bacteria. EDX analysis of encrusted colony shows Ca precipitation.

high density materials suggesting iron compounds which sporadically cover the sheath. Mn-Fe rich brown microbial mats sampled in Kanazawa form rapidly doughnut-shaped colonies on slide glass by *Bacillus* bacteria within a few days. Brown-black precipitates surrounding bacteria exhibit a radially growing pattern. The doughnut-shaped colonies occur as high dense of opaque materials covering a center-hole (Fig. 8).

**Electron microscopic observations**

Detailed examination of microbial mats were carried out by phase microscopy and electron microscopy using SEM and TEM to clarify their micro appearance.

**Siliceous spherules ;**

Direct examination of *Synechocysts* sp. by SEM revealed that the filaments were covered with abundant spherical precipitates, which were frequently observed to encrust the filaments completely. A SEM-EDX micrograph shows Si-O rich spherules in microbial mats in Geyser, Iceland (702-5 A) (Fig. 3). The spherules range 0.5-1 $\mu$m in diameter and aggregate to form colonies. TEM micromorphology (702-5 A) of thin-section of cyanobacteria, $>2\mu$m in diameter, is shown in Fig. 4 A (upper parts). Coccus or rod-shaped cyanobacterium, about 0.5-1 $\mu$m in diameter, is shown in Fig. 4 A and B. Colonial *Gloeothece* sp., $>50\mu$m in diameter, is also observed in the same sample. Bacterial cells are connected to develop a chain structure of *Nostoc* sp. The cell surfaces are covered with thick materials (Fig. 4). The initial precipitates surrounding the cell walls are identified to be amorphous siliceous materials by EDX analysis and XRD data. The formation of spherical silica precipitates by bacteria was observed both extracellularly and intracellularly (Fig. 4, arrow heads). Large spherules, about 200 nm in diameter, are also aggregated with siliceous materials.

EDX analyses of coccus cyanobacteria obtained from Abashiri in Japan show that the cell wall is completely encrusted with siliceous materials with high amounts of Si with S, Cl and Ca. Traceable amounts of Al, K and Fe are present as indicated by the characteristic peak pattern (Fig. 5). The elements of S, Cl and Ca are due to original components of

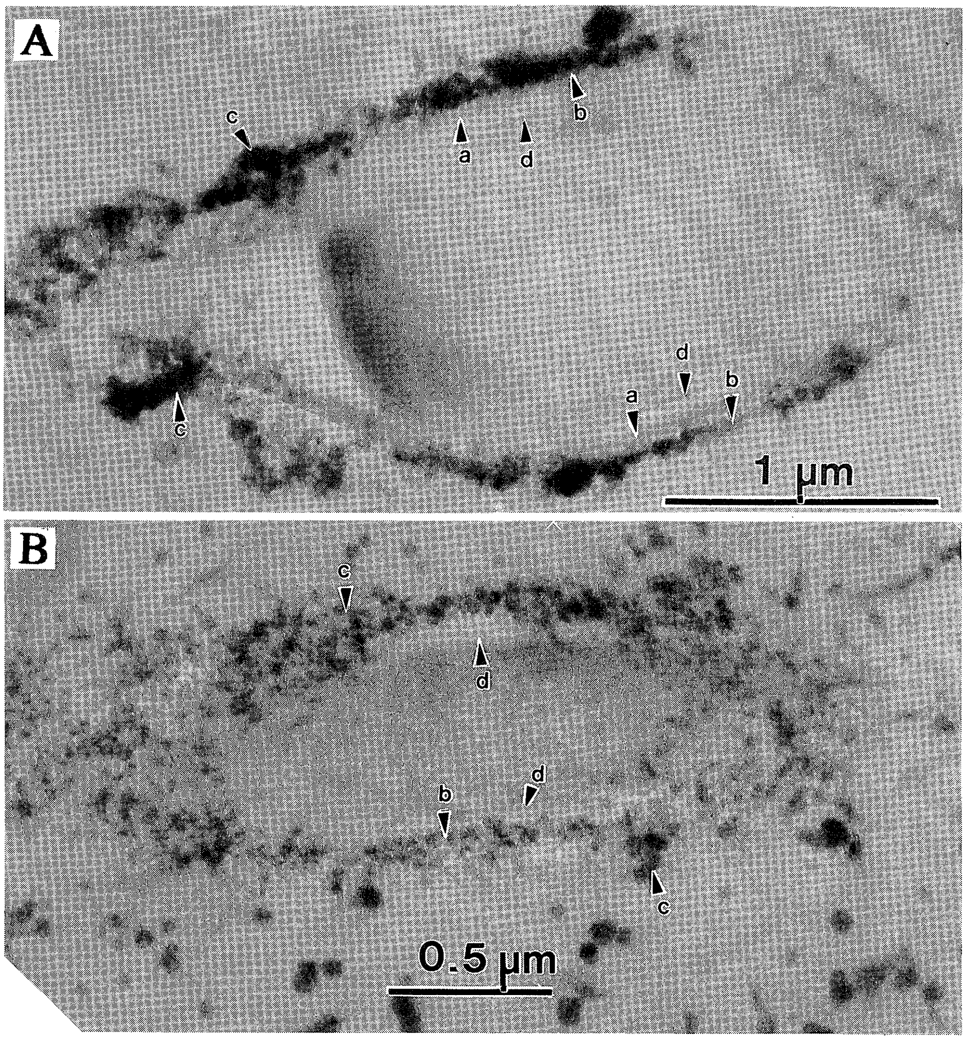



**Fig. 7.** TEM ultra-thin section micrographs of amorphous Fe (A) and Ca (B) concentrated spherules from Laugrvatn (703-5 C) showing internal cell structures.
a ; cell wall, b ; surface array, c ; capsule, d ; plasma membrane.

green living cyanobactrial body. Small spherules surrounding cyanobacteria, 2–3$\mu$m in diameter, are composed of Si suggesting cristobalite which was identified by XRD.

**Carbonate spherules ;**

SEM and TEM observations of the thin sectioned samples of the microbial mats in hot springs from Iceland have revealed intracellular structure and carbonates mineralization. *Cynechococcus, Synechocystis* and colonial *Gloeothece* are completely encrusted on the growing surface of the mats as shown in Figs. 6 and 7. Colonial bacterial cells are embedded in a pocket of carbonate (Fig. 6). The precipitates of microbial minerals were observed both extracellularly and intracellularly (Fig. 7). The thin section has revealed an evidence that biominerals can be grown in living cells with binary and nuclear structures (Figs. 6 B and 7). Several cyanobacteria in a colony might be living during the formation of encrusted colonial wall (Fig. 6 B). The intracellular structures of plasma membrane (d) facing surface array (b) and cell wall (a) are preserved inside capsule (c), even though the examined specimen was dehydrated to some extent (Fig. 7 arrow heads). Amorphous iron (Fig. 7 A) and carbonates (Fig. 7 B) are accumulated on both external cell and intracellular surfaces (Fig. 7). The well-developed large spherules on the cell wall are identified to be calcite by XRD of the selected part rich in spherules.

**Mn-spherules ;**

It was found in Kanazawa that in situ Mn-mineralization of bacteria occurred rapidly in river freshwater in normal temperature condition. As a result of the observation using optical and TEM microscope, the most common bacterium associated with Mn deposition proved to be *Leptothrix discophora*-like one (Fig. 8, 10 A). The doughnut-shaped holdfasts have formed large colonies which were identified as amorphous manganese by XRD (Fig. 2), SEM-EDX (Fig. 9) and electron diffraction (Fig. 10 B in set). The Mn-precipitating iron bacterium forms the filaments adhering rods within an enclosing sheath (Figs. 10 and 11). TEM micrographs show capsuled bacteria which are associated with extracellular and intact bacterial cells. Some empty capsules are found with films as shown in Fig. 11 A. Small spherules, 30 nm in diame-

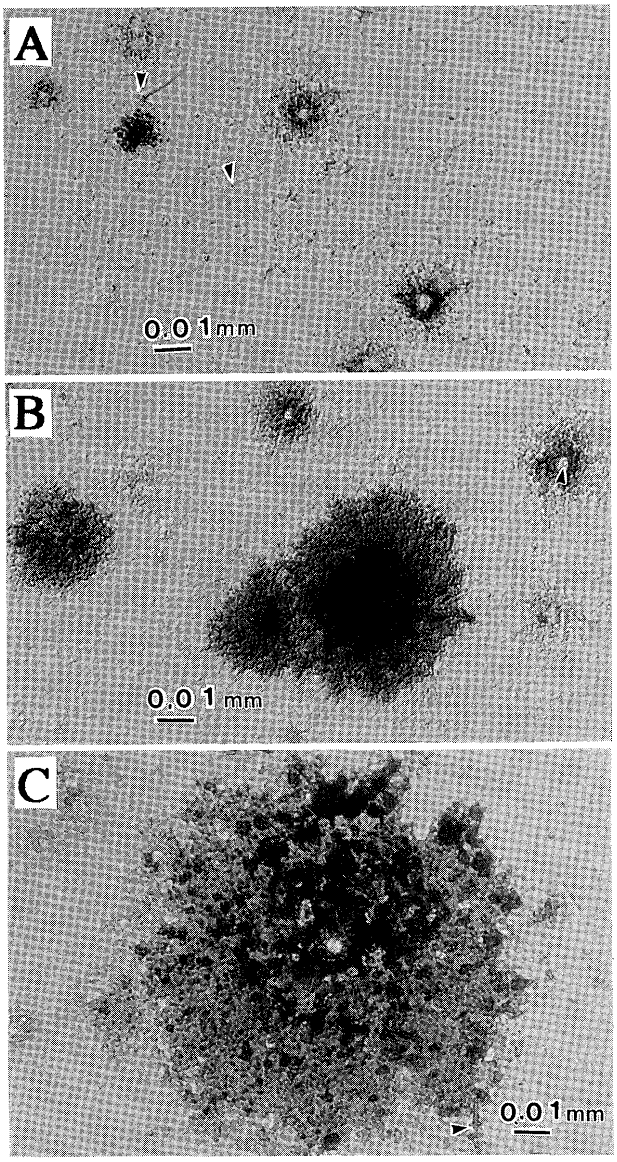



**Fig. 8.** Phase contrast light photomicrographs of *Leptothrix discophora*-like bacteria showing doughnut-shaped Mn-rich holdfasts. The holdfasts were enlarged and thicken to C from A.

ter, were observed to occur at incipient stages of amorphous manganese formation (Fig. 11 B). In many filaments, the Mn crystallites appeared to merge, so that individual bacterial cell might be no longer distinguishable. Filamentous materials were connected with the small spherules to make nets.

In the hot springs of Sokcho in Korea, well crystallized buserite was produced biologically. High resolution TEM micrographs of the films surrounding bacterial cells show 7.5 and 3.5 Å lattice images (Fig. 12 A, B) suggesting probable buserite d-spacings. XRD data of the microbial mats after heating (Fig. 2) also support the presence of buserite. The films are identified to be composed mainly of Mn with traces of Ca and Si by EDX (Fig. 12).

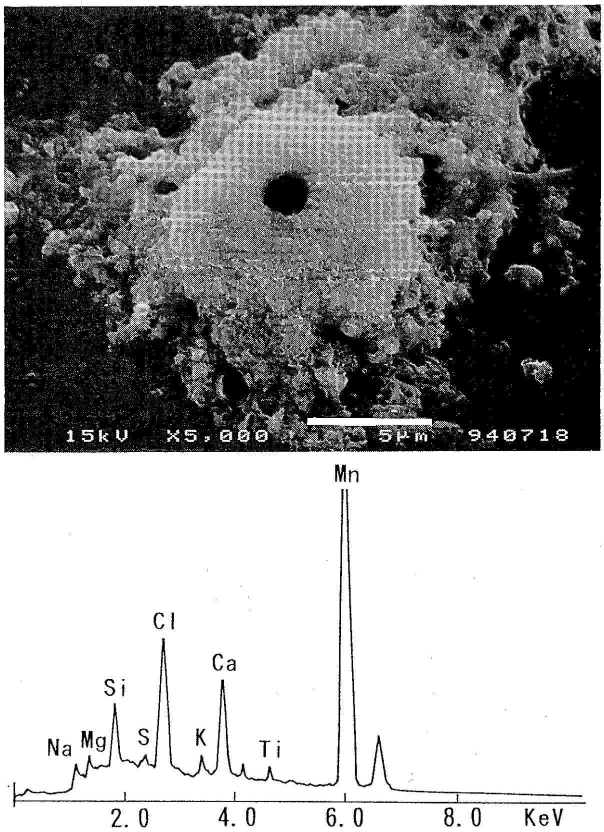



**Fig. 9.** SEM micrograph and EDX of Mn-rich doughnut-shaped holdfast from freshwater in Kanazawa. XRD data showed amorphous materials.

**Fe-spherules ;**

Fe-precipitating iron bacteria (Fig. 13 arrow heads) in bright orange microbial mats were observed in the freshwater system in Kanazawa. The colonies form doughnut-shaped holdfasts. The spherules are mainly composed of Fe with Na, Al, Si, K and Ca (Fig. 13). River water contains high concentrations of dissolved Fe, Si and Mn (Tazaki et al., 1995). The sheaths of *Leptothrix ochracea*-like bacteria form spherules, 50–200 nm in diameter (Fig. 14). The holdfasts of bacteria are recognized in the early stage (Fig. 14 A) of advanced state (Fig. 14 B). The bacteria grew as filaments, which consisted of a long chain of cells growing within a cylindrical, tube-like sheath made up of fine spherules. The initial precipitates had a spherical morphology and consisted of amorphous iron, as indicated by EDX. In the late stage of preservation, chain structure is perfectly formed, having several spherules with high density (Fig. 14 C). As the remains of cells became completely embedded, wall structure was eventually lost, leaving behind a "cast" of the bacterial filament similar to microfossil assemblages. Electron diffraction patterns of these spherules indicate that those are amorphous hydrated iron oxides (Fig. 14 C, inset). The spherule is composed of granular grains as shown in the upper part of Fig. 14 C.

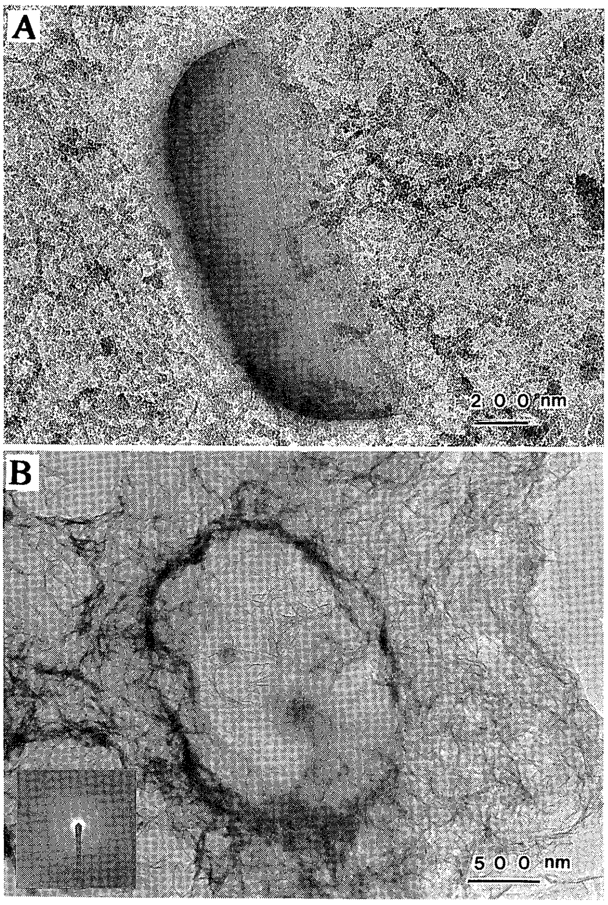



**Fig. 10.** Transmission electron micrographs of Mn-rich doughnut-shaped holdfast from freshwater in Kanazawa. XRD data showed amorphous materials. A ; living bacterial cell on granular matrix, B ; preserved bacterial cell wall precipitating amorphous Mn-thin films. The electron diffraction pattern shows diffuse rings (inset).

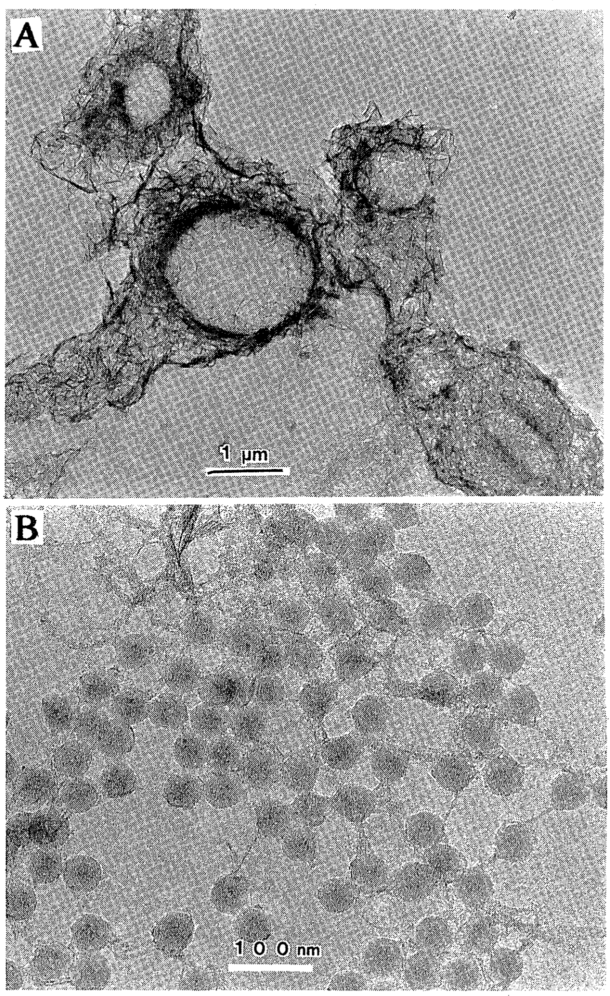



**Fig. 11.** Transmission electron micrographs of Mn-rich doughnut-shaped holdfast from freshwater in Kanazawa. A ; preserved bacterial cell walls and empty cells were cupcelled by Mn-thin films, B ; small spherules were spreaded with filamentous net work structures.

## Discussion

In this study, XRF, XRD, optical and electron microscopic observations have revealed elemental concentrations and biological mineral formations on cyanobacteria in the microbial mats sampled from hot springs and river freshwater systems. The microorganisms exhibit a remarkable ability to concentrate Si, Ca, Mn and Fe in various pH and temperature conditions. Formation processes of microbial silicates, carbonate, manganese and iron oxide minerals are clearly observed in TEM micrographs of bacterial internal and external cell walls. Ultra-thin section of a bacterium has revealed various surface structures, such as plasma membrane, cell wall, surface array and capsule. Membrane surrounded by an electron-transparent cortex is composed of peptido-glycan and outer proteinaceous coat layers (Doyle and Marquis, 1994). A portion of a bacterium cell is surrounded by dark colored cation complex composed of enzymes, polymer biominerals. Bacterial surface with minus charge is often easily used as a nucleation site for cation and hence grown as authigenic minerals. The consistent formation of minerals by all bacterial populations, regardless of substratum lithology, implies that biomineralization was a surface process associated with the anionic nature of the cell wall. Once completely capsuled and split, the resulting poles become rounded and spheruled due to cellular turgor pressure (Schindler, 1993 ; Kozel, 1995). Microbial mats are densely entangled with their mineral substrate.

A schematic illustration of the elemental cycles related to bacterial mineralization is shown in Figs. 15 and 16. TEM showed that attached bacteria (on all substrata) were highly mineralized, ranging from Fe-rich capsular material to fine-grained authigenic (primary) mineral precipitates. Mineral formation remains localized in the sheath until after cellular

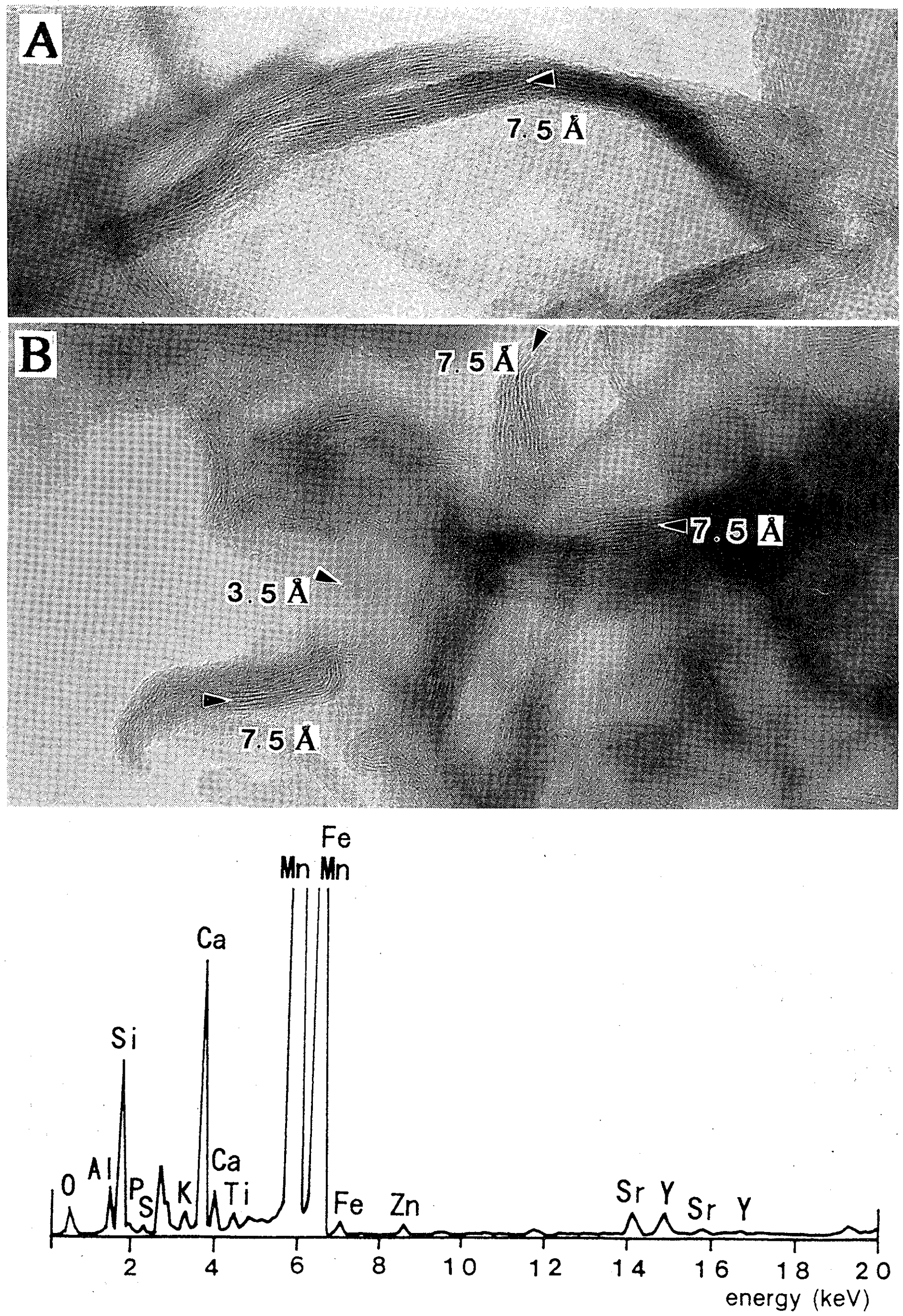



**Fig. 12.** High resolution transmission electron micrographs of well crystallized buserite from Sokcho, Korea, showing 7.5 and 3.5 Å lattice images on the thin films. EDX shows strong Mn peaks associated with small Si and Ca peaks.

death (Fig. 15). While the cells remain metabolically active, mineralized sheath materials are shed and replaced by new materials. Silica crystallites are formed by subsequent hydrolysis and polymerization of the bound silicic acid (Fig. 16). The adsorption of dissolved constituents from the aqueous environment contributed significantly to the mineral formation process. Schindler (1993), Doyle and Marquis (1994), and Mobley and Belas (1995) have reported on bacterial cell wall properties, and emphasized that the peptidoglycan sacculus serves as a mechanical framework for the cell walls of most eubacteria and largely determines cell shape. Accordingly the mechanical structure of producing a rigid shell is still a matter for a full discussion, because a rigid shell is contradicted by findings that peptidoglycan and/or polypeptide can expand or contract (Jenkinson, 1995). Thus, the sacculus performs an elastic, flexible, polyionic, amphoteric, and restraining network (Doyle and Marquis, 1994). It may appear chaotic in the details of its structure but it can be accurately described by simple models for fractal geometry (Ohgiwari et al., 1992 ; Schindler, 1993 ; Nakahara, 1995).

**Si concentration :**

The mechanism of Si concentration and silicification in the fossilization processes has widely been discussed (Urrutia and Beveridge, 1994 ; Tazaki, 1994, 1995 b ; Schultze-Lam et al., 1995). Silicon-uptake

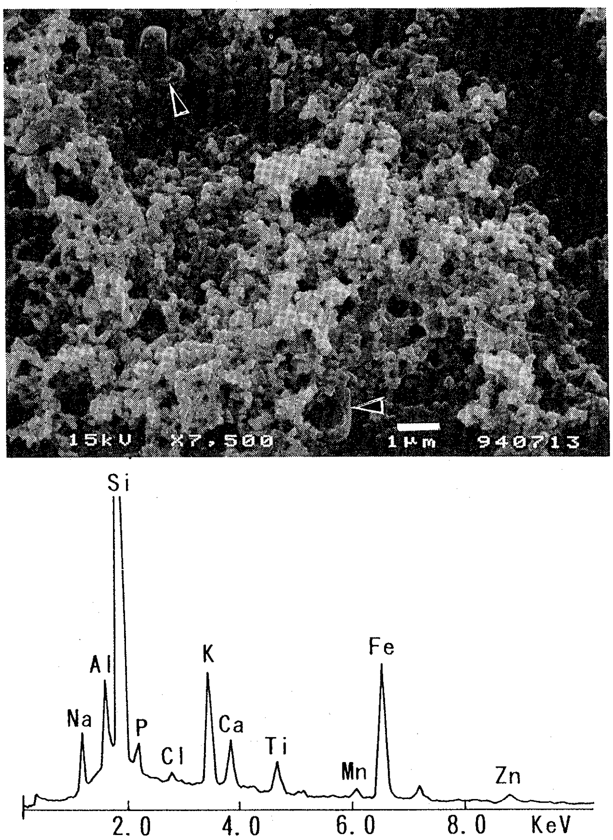



**Fig. 13.** SEM micrograph of Fe-rich spherules with bacteria (arrow heads) and EDX, from freshwater in Kanazawa.

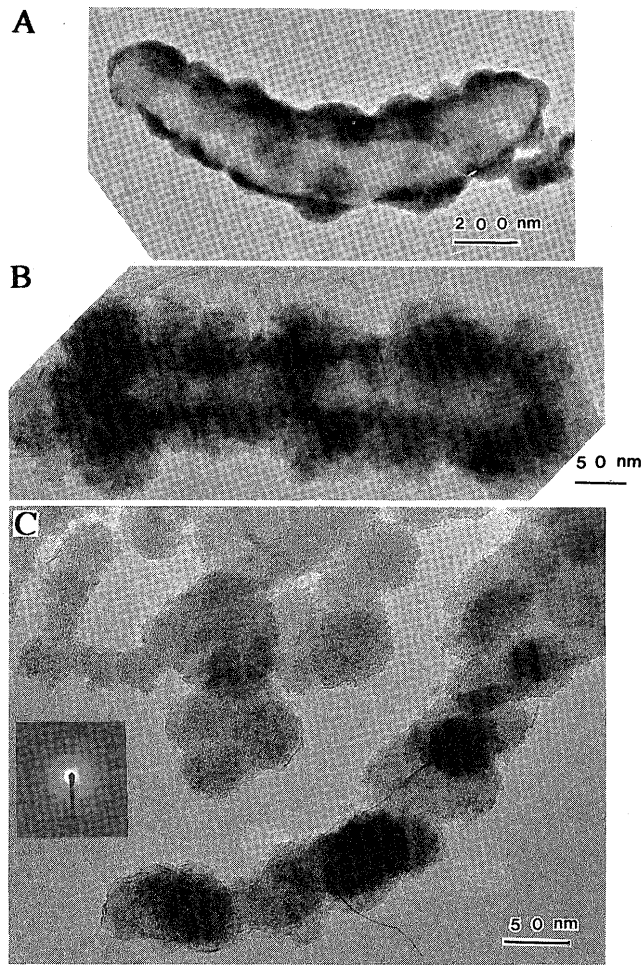



**Fig. 14.** Transmission electron micrographs of cyanobacteria in freshwater in Kanazawa, showing amorphous Fe-deposition processes from A (at an initial stage) to C (at a developed stage) on the cell surface.

and deposition by microorganisms in hot springs and rivers are compensated by dissolution from cell walls after death. In this study, the mineralization of thick crusts of microbial siliceous materials on/in living cell has been focused on hot springs. The uptake and incorporation of silicon into cells suggests that the aquatic microbiology influences the authigenic siliceous mineral formation. Below pH 9, silicon is generally released as weak monosilicic acid ($H_4SiO_4$). Bavestrello et al. (1995) reported that quartz particles were strongly etched and made uniform in size by a marine organism with remarkable selectivity. These results in the production of silica casts of bacteria, which bear a striking resemblance to the microbial remains in ancient microfossil assemblages. Although the rock substrate differed extensively from granites, sandstone, limestone, and concrete, the epilithic bacteria consistently formed (Fe, Al) silicates (Konhauser et al., 1994). Rock lithology has limited the influence on an authigenic mineral formation implying that biomineralization was a surface process, associated with the anionic nature of bacterial cell walls.

**Ca concentration :**

Ca-concentrated bacteria are commonly found in the environment of many hot springs. The formation of calcite on cyanobacterial cell wall shows the importance of nucleation sites for Ca deposition (Freiwald, 1995 ; Vasconcelos et al., 1995 ; Tazaki, 1995 a). Most of the $CO_2$ fixed by plant biomass are quickly respired back into solution, either by organisms themselves or by animals and bacteria after consumption (Ducklow and Fasham, 1992). In this study, the formation of thick crusts of microbial calcite or amorphous carbonate on cell walls was observed in hot spring seeps in alkaline conditions. The results suggest that the deposition of $CaCO_3$ is apparently caused by ion movements to the cell interior from the outside solution at the different stages through growing process. Amorphous carbonate is deposited on the cell surfaces, and then organized into more crystalline structures of calcite. Generally cyanobacterial calcification has been considered to be primary by the extracellular nucleation of calcium carbonate (Leadbeater and Riding, 1986). Ammonia is oxidized to nitrite ($NO_2^-$) by cyanobacteria which are capable of the fixation of $CO_2$ and $HCO_3^-$ during photosynthesis (Chapelle, 1993).

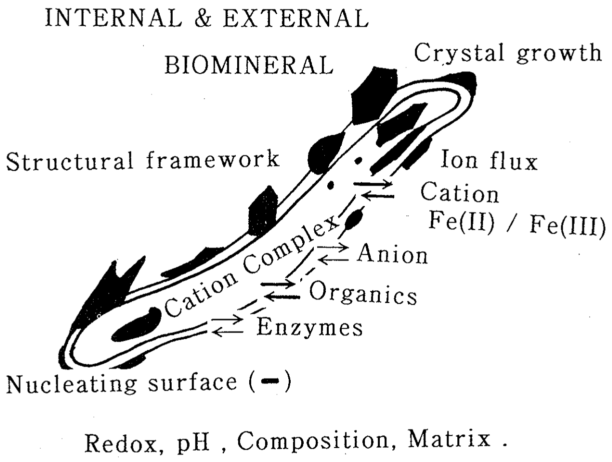



**Fig. 15.** A schematic diagram of the bacterial biomineralization at internal and external surfaces.

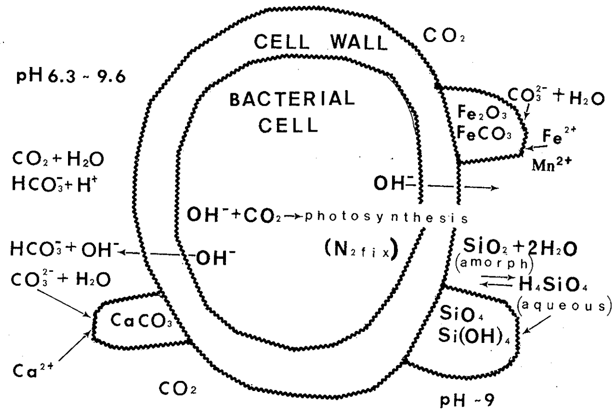



**Fig. 16.** A schematic illustration to explain precipitation process of Si, Ca, Mn and Fe ions on bacterial cell surface.

The formation of $CO_3^{2-}$ ions involved in $CaCO_3$ formation is almost instantaneous. However, according to Mann et al. (1989), above pH 9, the dehydration of $HCO_3^-$ to $CO_2$ may become rate limiting to photosynthetic $CO_2^-$ uptake if the algae can only take up $CO_2$. When $CO_2$ is taken up by the cell during photosynthesis, this can lead to localized alkalinization of the medium near the cell ; the rise in pH then results in an increase in the concentration of $CO_3^{2-}$ (Mann et al., 1989). The $CO_2/HCO_3^-/CO_3^{2-}$ equilibrium changes in pH is brought about by $CO_2$ uptake. Thus, the rise in pH due to photosynthetic uptake of $CO_2$ will become the greatest in water at pH of about 8. The $CaCO_3$ mineral is formed from $HCO_3^-$ moving out from the cell extensions, then protons will be released into the cuticle meshwork. If these protons were to accumulate, further precipitation of $CaCO_3$ would be inhibited. The hydration and dehydration of carbon dioxide is catalyzed by carbonic anhydrase (Ducklow and Fasham, 1992). Early in the earth's history Ca-microbial mats may have had a significantly higher proportion for oxygenation of the atmosphere.

**Fe and Mn concentrations :**

Natural waters commonly contain fine grained iron and manganese compounds, suggesting the interactions of microbes with iron and manganese. Ferrihydrite is one of the biomineral components in biota (Mann et al., 1989 ; Lowenstam and Weiner, 1989 ; Stanier et al., 1986 ; Tazaki et al., 1994 ; Konhauser et al., 1994). Manganese oxide, buserite, has been found not only in hot springs but also in freshwater (Tazaki et al., 1994). In general, iron bacteria are important in the accumulation and deposition of iron and manganese in the local environment (Krumbein, 1979 ; Simkiss and Wilbur, 1989 ; Lowenstam and Weiner, 1989 ; Tazaki et al., 1992 a,b, c, and 1993 ; Mann et al., 1992 ; Skinner and Fitzpatrik, 1992). Fe-Mn microbial mats are organized in response to the sediments by the products of biotransfer.

In this study, SEM-EDX and TEM observations have revealed that cyanobacteria have chemically, mineralogically and morphologically distinct Fe and Mn biominerals on the cells. The micron-scale analytical investigations were of extreme importance in elucidating the elemental distribution which modifies the biologically deposited iron and manganese. Microbial reactions producing compounds such as $Fe_2O_3$ and $FeCO_3$ and the interplay between the biota and iron are environmentally significant because of the fixation of carbon dioxide in the carbon cycle (Fig. 16). Ferris et al. (1988) have shown that cellular preservation occurs through silicification. Because the higher concentrations of dissolved silica in modern-day aqueous surface environments are found in hot springs, it is likely that some microfossils developed in similar environments. The main mat-forming organisms are usually filamentous bacteria, which include *cyanobacteria* and other phototrophic bacteria that have often associated with mineral precipitates (Ferris et al., 1986).

High concentrations of dissolved iron (Fe (II)) are very common in anaerobic ground-water systems. According to Chapelle (1993), Fe (III) reduction was considered to occur spontaneously and reversibly. Particular microorganisms were able to take advantage of this process in order to obtain some energy for growth. Microorganisms obtain energy by iron reduction, and this reaction appears to produce iron oxide minerals and to fix $CO_3^{2-}$. Microorganisms exist which are capable of producing $FeCO_3$ during $CO_2$ fixation. Iron probably precipitates as a proto-ferrihydrite and replaces the mobilized manganese (Pracejus and Boiton, 1992 ; Sawicki et al., 1995).

Iron ions are easily substituted manganese ions by microorganisms, although depending on Eh-pH conditions (Pracejus and Boiton, 1992). Many of secondary manganese minerals are highly oxidized and occur as tetravalent oxides (Larock and Ehrlich, 1975 ; Cowen and Bruland, 1985 ; Cowen et al., 1986). However, lower valent Mn minerals may have played an important role in the early history of the deposit. In natural systems the only significant and dissolved Mn species are divalent ionic forms. At a pH$<$7.5 in river water, essentially all manganese seem to be present as the reduced $Mn^{2+}$ species (Schmidt and Robbins, 1992 ; Robbins et al., 1992 ; Tazaki et al., 1995). Generally, Eh/pH stability ranges for manganese oxides and hydroxides become relatively small when we use the highest concentration of dissolved manganese detected in the field. In the reaction, manganese oxides and dissolved iron exchange electrons, and manganese are reduced from $Mn^{4+}$ to $Mn^{2+}$ (or Mn (OH)). $Mn^{4+}$ mineral formation often occurred at pH 7–8 in the variety of conditions, such as that $Mn^{2+}$ concentrations are too high to be favorable for the disproportion of $Mn_3O_4$, or $\beta$ MnOOH to $Mn^{4+}$. Direct oxidation of $Mn^{2+}$ to $Mn^{4+}$ by microbes is a common process in the natural environments (Mandernack et al., 1995 a ; Mandernack et al., 1995 b). Organic-rich layers are formed by cyanobacteria and other type of filamentous bacteria, and authigenic mineral precipitates will remain in detrial inorganic sediments beyond the death of bacteria into the future.

## Conclusions

The microorganisms exhibit a remarkable ability to concentrate Si, Ca, Mn and Fe spherules in the various pH and temperature conditions. The formation processes of microbial authigenic silicates, carbonate, manganese and iron oxide spherules are clearly shown in the TEM micrographs of the bacterial internal and external cell walls. Ultra-thin section of an individual of bacteria has shown the various surface structures, such as plasma membrane, cell wall, surface array and capsule. Membrane is composed of peptido-glycan and outer proteinaceous coat layers. Bacterial surface with minus charge is easily offered nucleation site for cation and authigenic minerals. Once completely capsuled and split, the resultant poles become rounded and spheruled due to cellular turgor pressure. Cyanobacteria play an active role in the deposition of authigenic minerals, and provide precipitation sites. Microbial mats are organized in response to the sediments by the biotransfer products which will ultimately become part of the sediments. Biomineralization of the interior bacterial cell, as described in this study, suggests an important prerequisite for preservation of cellular structure in the geological record.

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**Tazaki, K. and Ishida, H., 1996, Bacteria as nucleation sites for authigenic minerals. *Jour. Geol. Soc. Japan,* 102, 866-878.**（田崎和江・石田秀樹，1996，バクテリアによる自生鉱物の核形成場．地質雑，**102**, 866-878.）

微生物の関与により元素の輸送や移動がおこり，その結果，堆積物中に自生鉱物が形成される．各地の温泉や河川に見られる微生物被膜を電子顕微鏡で観察すると，バクテリアの細胞の内外に，球粒の自生鉱物が認められた．それらは，非晶質，低結晶質，高結晶質の炭酸塩鉱物，珪酸塩鉱物，Mn-Fe 酸化鉱物であった．微生物被膜の中に生息するバクテリアは，自生鉱物の核形成の場を与え，バクテリアの死後，自生鉱物は堆積物へと移行する．