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学位授与の題目	Silica-biomineralization of the unicellular red alga, *Cyanidium caldarium*, in acidic hot spring and in laboratory cultivation vessels（現地観察と培養実験による出湯小米（イデユコゴメ）のSi－生体鉱物化作用）
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学位論文要旨

Abstract

In this paper, the acido- and thermo-philic unicellular red alga, *Cyanidium caldarium,* a common member of microflora in green biomats at acidic hot springs, Japan was studied. Green biomats are widely distributed over the face of the falls, around the spouts of hot spring, along the margins of hot-spring pools and along outflow channels under the acidic (pH 1～3) and high temperature conditions (30～60 ℃). Most unicellular microbes in green biomats have silica crusts on the cell surface. The crusts are amorphous silica / low crystallinity of silica. Double layered silica crusts are often found, suggesting that the microbes have high tolerances for acid and temperature. The colonies of *C. caldarium* cells have gradually changed into angular shape with concentration of silica. When the colony developed, the part of the inside decolorized into transparent color due to lose chlorophyll. Microbial cultivation experiments revealed that silica crusts had positive effects for viability of *C. caldarium* and that distribution of microbes and microbial community were associated with the formation of banded biomats.

***Key words*:** biomineralization, biomats, silica crust, *Cyanidium caldarium,* cell wall, acidic hot spring

Silicon (Si), one of the most abundant elements in the earth's crust, exists in various states such as silica minerals, silicate minerals and amorphous silica ions, and occurs in various circumstances of the Earth's surface in igneous and sedimentary rocks, underground water, hot spring water, and bodies of organisms. Therefore, silicon is extremely important from its position in the periodical system of elements, from its abundance and its importance in man-made and natural substances used by human society.

Silica-biomineralization in biomats was described from macro to micro scale morphology and chemistry in this paper. Silica-biomineralization occurs at green biomats of three areas, Kamuiwakka Falls, Hokkaido, Japan, Higashi Hot Spring, Satsuma-Iwo Jima Island, Kagoshima Prefecture, Japan and Fukiage Hot Spring, Hokkaido, Japan. Green biomats forming under strong acidic conditions ($pH < 2$) consist mainly of the acido- and thermo-philic unicellular red alga, *Cyanidium caldarium,* a common member of microflora in acidic hot springs worldwide.

C. caldarium cells are green colored and spherical in shape. Electron diffraction patterns also showed that the mineralogical structure of the colony in green biomats is amorphous silica / low crystallinity of silica. SEM observation of the surface of green biomats revealed that most unicellular microbes have crusts on the cell surface. Numerous granular particles attached to the cell walls which form completed crust(Fig. 1). EDX analysis showed that the crust was composed of a lot of Si with a little S and Cl (Fig. 1). TEM observation of ultra thin-sectioned unicellular microbes, *C. caldarium* revealed the relations among silica crusts, cell walls and cytoplasm (Fig. 2).

The colonies of *C. caldarium* cells have gradually changed into angular shape with concentration of silica, and simultaneously decreased the amount of organic matter with P and S contents. When the colony developed, the part of the inside decolorized into transparent color due to lose chlorophyll. Natural cultivation examination of *C. caldarium* indicated that the development of the colony depended on solution pH, nutrients, silica-concentration, growth rate of the cells and eco-system with bacterial condition, and played an important role in silica-biomineralization.

Silica crusts containing dead cells remained in-situ as white biomats. The inter spaces between silica crusts were progressively filled with silica. The mineralogical structure of white biomats transformed amorphous silica from cristobalite. Electron microprobe elemental images of a polished section of white biomats revealed that the biomats had a layered structure. Si X-ray map revealed that high intensity of silicon (Si) had positive correlation with high intensity layer of S X-ray map. While, Fe X-ray map, the high intensity showed negative correlation with high intensity layer of S X-ray map. The complexes of silica and microbes in green biomats can support a long-standing supposition that such species play a significant role in the biological uptake and transport of silicon and in mineral diagenesis. The formation processes of white biomats were discussed.

Microbes associated with *C. caldarium* living in green biomats were also distributed. To understand the ecology of the microbes living under strong acidic condition (pH < 2), the microbes containing unicellular microbes, *C. caldarium* were cultured. Various symbiotic microbes appeared when they cultivated in deferent conditions. In this study, the activities were macroscopically investigated in microbial cultivation systems without sediments and rocks. The facts indicate that silica crusts have positive effects for viability of *C. caldarium.*

Silica-biomineralization and biomats formation by *C. caldarium* cells and microbes associated with *C. caldarium* in acidic hot springs and laboratory cultivation vessels were discussed in this study.

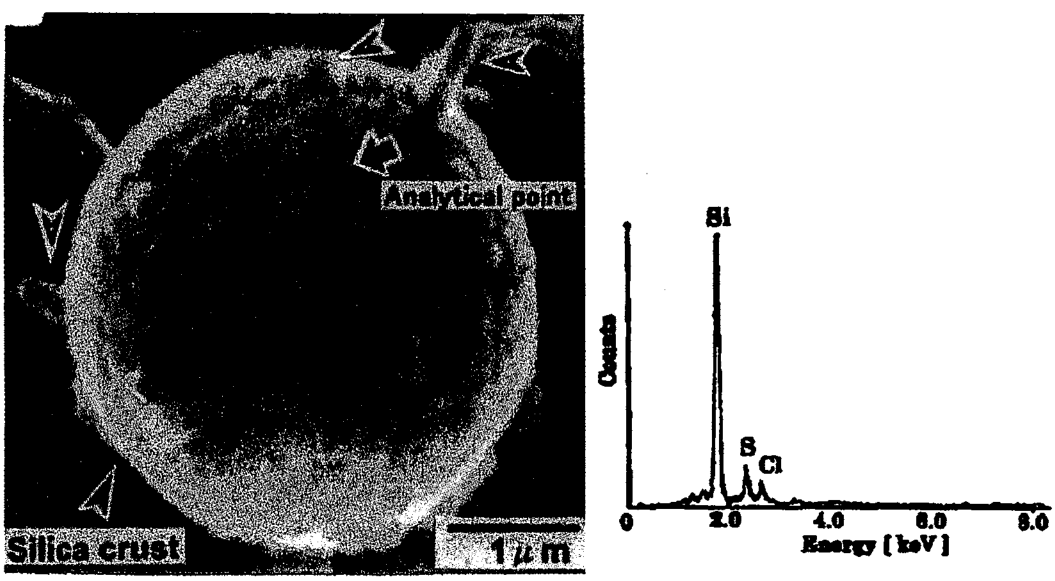

Fig. 1 SEM micrograph of unicellular microbe, *Cyanidium caldarium,* Kamuiwakka Falls showing silica crusts on the cell walls. A crust with numerous granular particles and projections (small arrows). EDX spectrum of the crust shows a strong peak of Si and weak peaks of S and Cl (Analytical point: thick arrow).

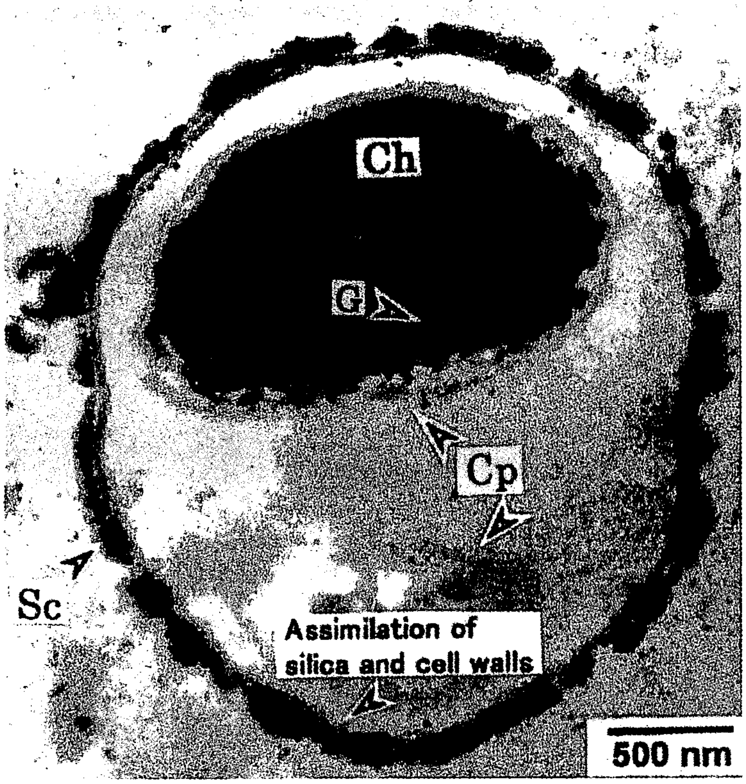

Fig. 2 TEM images of ultra-thin-sectioned unicellular microbes with flexible silica crusts. Cytoplasm (Cp) is often far from the silica crusts and then granular silica particles are not found on the surface. A single layered silica crust assimilates the outer cell wall. Ch: chloroplast, G: granular particles, Sc: silica crust.

学位論文審査結果の要旨

本学位論文は全国各地の酸性泉に生成する緑色バイオマット中の好熱・好酸性単細胞紅藻類イデユコゴメ(出湯小米, *Cyanidium caldarium*)について研究を行った。現地観察の結果，イデユコゴメはpH1～3，温度30～60℃の環境下で生息し，温泉の湧出口付近，温泉が流れる斜面や浴槽の縁などによく発達する。また，緑色バイオマットの光学顕微鏡・電子顕微鏡観察により，その細胞壁は非晶質あるいは低結晶質シリカが析出しており，細胞壁全体を覆い殻(シリカクラスト)を形成していることが明らかになった。そのシリカクラストは2重になっていることが多く，イデユコゴメの耐熱・耐酸性の能力を高めていると考えられる。また，イデユコゴメのコロニー形成に伴い，その内部にシリカの濃集および結晶化が生じ，コロニー全体の形態が角張ってくる。さらに，イデユコゴメ内部の葉緑体の溶出による透明化がおこることが明らかになった。温泉水を用いた培養実験において，シリカクラストがイデユコゴメの生存能力に効果をもつことおよび微生物の分布やコミュニティーがそのバイオマットの層構造の形成に大きく関っていることが明らかとなった。本論文では「生命と鉱物」の境界領域の分野において，貴重なデータを提供した。参考論文は国際誌に掲載(2月号)が決定しており，海外における評価も高い。以上五名の審査員はいずれも朝田隆二の論文が学位に相当する質の高い論文であると評価した。