Biomineralization in natural environments : the effect of microorganisms inhabiting hot spring water and biomats on mineral formation

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学位論文要旨

Abstract : Bacterial mineralization or biomineralization is a common and widespread phenomenon occurring in various geothermal systems. An *in situ* visualization of the fossilized microorganisms in the green biomats and deposits of the Kotelnikovsky hot springs (Lake Baikal, Russia), allowed concluding that silicification and calcification of the microorganisms extensively appeared in the biomats and deposits under pH 8.0 hydrothermal conditions. The thermal water of the Vilyuchinskie springs, Kamchatka peninsula, Russia, showed the oxidative effect of the oxidation-reduction potential, had a temperature of 56°C, a neutral pH and high contents of iron and silicic acids. Ferric iron predominated over the ferrous form at a ratio of 52:7 in the brown biomats. The accelerated processes of iron oxidation, silicification, and deposition of Fe-Si minerals that occur in the brown biomats can be attributed to the impact of rod-shaped bacteria inhabiting the hot spring water and biomats. A positive correlation between the activity of Fe-bacteria in the water, their presence in the biomats, and the chemical content of water and deposits was found for Oksinskie, Nalychevskie, and Dachnye hot springs (Kamchatka peninsula, Russia). In chemolithotrophic environments, the microbial abundance and the types of bacterial activities reflect the biological components of the biogeochemical

processes occurring in the ecosystem.

Bacterial mineralization or biomineralization is a common and widespread phenomenon occurring in various geothermal systems. The important role of microorganisms in the formation of modern hydrothermal deposits was denoted recently. Bacteria are very small (~ 1.5 μm^3), but have the largest surface to volume ratio of any life form. Therefore, they provide a large contact area that can interact with metals in the surrounding environments. Microbial activity is responsible for the transformation of at least one third of the elements in the periodic table. The main objective of the present study was to investigate hot spring water, biomats and deposits in order to observe microorganisms that could be actively involved in biogenic mineral formation and were associated with mineral particles.

Samples of water, green biomats and modern hydrothermal deposits were collected from two sampling areas namely Lake Baikal, Siberia and Kamchatka peninsula, Russia. Physicochemical variables of the hot spring water [pH, oxidation-reduction potential (Eh), electrical conductivity (EC), dissolved oxygen (DO), water temperature (WT)] were measured in the field. Water, biomats, and deposits were analyzed chemically with energy dispersive X-ray spectrometer. Mineralogical composition of green biomats and deposits was identified with X-ray diffractometer. Microscopic observation of bacteria inhabiting hot spring water and biomats was done with optical microscopy. Fossilized microorganisms in green biomats and deposits as well as dominant bacterial species in hot spring waters were studied with scanning and transmission electron microscopy equipped with energy dispersive X-ray spectrometer. Additionally, thermophilic microorganisms isolated from water and biomats of Kotelnikovsky, Khakusy, and Zmeinaya hot springs (Lake Baikal, Russia) and consortium of culturable iron-oxidizing bacteria from Vilyuchinskie hot springs (Kamchatka peninsula, Russia) were studied in laboratory experiments.

Activity of microorganisms was estimated *in situ* in natural samples of hot spring waters and for culturable thermophilic bacteria.

Alkaline and neutral pH for Baikal and the Kamchatka hot springs, respectively, were revealed during measurements of physicochemical variables. Low values of dissolved oxygen and high water temperature were mainly determined. General feature of chemical composition of hot spring waters is high content of Si. Calcium and silicon were detected in high content in deposits as well as in green biomats from the Kotelnikovsky hot springs, while, iron and silicon were found mainly in the Vilyuchinskie hot springs. Additionally, biomats were enriched in bio-elements such as potassium, magnesium, iron, phosphorus, and sulfur.

An *in situ* visualization of the fossilized microorganisms in the green biomats and deposits of the Kotelnikovsky hot springs located on the northwest coast of Lake Baikal, Russia, allowed concluding that silicification and calcification of the microorganisms extensively appeared in the biomats and deposits under pH 8.0 hydrothermal conditions. The biologically induced precipitation could be proposed for calcite formation in the hot spring waters, whereas, biologically controlled mineralization of Si-Ca materials could be denoted for green biomats and deposits. The essential role of silicate ions has been proposed for the formation of amorphous structures which are rich in calcium.

The culture collection of culturable thermophilic bacteria isolated from water and biomats of Kotelnikovsky, Khakusy, and Zmeinaya hot springs includes totally 91 strains which were grown at 53°C. Most of isolated strains were gram-positive spore-forming rods and belonged to genus *Bacillus*. It was denote that the highest fraction of strains showed higher degree of variable enzymatic activities rather at temperature of 50°C than that at 20°C.

The thermal water of the Vilyuchinskie springs, namely V3 site, Kamchatka

peninsula, Russia, showed the oxidative effect of the oxidation-reduction potential, had neutral pH, water temperature of 56°C and a high iron concentration. Ferric iron predominated over the ferrous form at a ratio of 52:7 in the brown biomats. Respiring iron-oxidizing bacteria were present in the water from the V3 spring and iron-bacteria were isolated from the biomats. In cultured iron-oxidizing bacteria, amorphous mineral particles were observed around the bacterial cells, suggesting that Fe(II) oxidation is catalyzed or mediated by these microorganisms. Moreover, the waters of the Vilyuchinskie springs are rich in silicic acid and there is a rapid rate of silicification during water discharge and cooling. The accelerated processes of iron oxidation, silicification, and deposition of Fe-Si minerals that occur in the brown biomats of the Vilyuchinskaya hydrothermal system can be attributed to the impact of rod-shaped bacteria inhabiting the hot spring waters and biomats (Fig. 1).

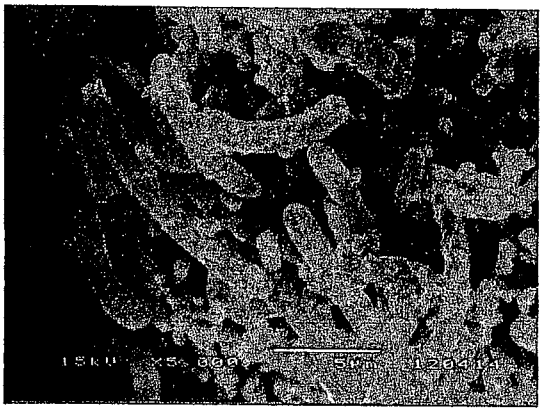

Fig.1. Scanning electron micrograph of rod-shaped bacteria inhabiting biomats of the Vilyuchinskie hot springs, Kamchatka peninsula, Russia, those are responsible for Fe-mineral formation.

Additional and very important data were obtained from bacterial enumerations. A positive correlation was found between the percent of CFDA+ cells and the pH of the waters. Respiring iron-oxidizing bacteria were observed in the water of the Oksinskie, Nalychevskie, and Dachnye springs, and growth of Fe-oxidizing bacteria was obtained on Fe-agar plates with inoculations from the Nalychevskie and Dachnye hot spring biomats. Thus, there is a positive correlation between the activity of Fe-bacteria in the hot spring water, their presence in the hot spring biomats, and the chemical content of hot spring water and deposits. It is important to point out that the metabolic activity of microorganisms, such as iron-oxidizing bacteria, can induce

mineral precipitation as a secondary by-product; a process referred to as biologically induced mineralization.

学位論文審査結果の要旨

平成16年7月15日に第1回学位論文審査委員会を開催し，7月28日に口頭発表を行い，同日最終の審査委員会を開催した．協議の結果以下のとおり判定した．本学位論文は，ロシア・バイカル湖畔のKotelnikovsky温泉及びロシア・カムチャツカ半島の4つの温泉（Vilyuchinskie，Oksinskie，Nalychevskie，Dachnye）についてそれぞれに生成する多様な温泉バイオマットを地球科学的また微生物学的な視点から研究し，生体鉱物化作用の特徴を議論したものである。バイカル湖のKotelnikovsky温泉の緑色バイオマット及び堆積物中の微生物の観察から，pH8.0以下の熱水条件下では微生物により蛍石と方解石が形成される．カムチャツカ半島のVilyuchinskie温泉は鉄と珪酸に富む中性の温泉である。ここでは，褐色バイオマット中の第一鉄:第二鉄の比が52:7でありフェリハイドライトが形成するが，これはバイオマット中の桿菌の酸化作用によると考えられる．カムチャツカ半島のOksinskie，Nalychevskie，Dachnye温泉では鉄バクテリアの存在およびその活性，温泉水と堆積物中の鉄の含有量の間には正の相関関係があることが明らかになった．これらの温泉環境下では，微生物の存在割合と微生物学的活性度が生体鉱物の形成に関与する．参考論文は二つが受理され印刷中である。以上5名の審査員はいずれも学位に相当する質の高い論文であると評価し，博士（学術）に値するものと認定した．