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原　　著

# Relation between Bacteria and Deposits found in the High $PCO_2$ Spring Waters of Primorye, Far East Russia

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# ロシア極東・プリモーリエ地区に点在する二酸化炭素分圧の高い冷鉱泉に生息する細菌と沈殿物形成への関連性

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## 要　　旨

ロシア極東・プリモーリエ地区はウラジオストックとハバロフスクにはさまれた広大な森林地帯を指し，そこには鉄を多く含む炭酸ガス分圧の高い冷鉱泉が多く点在している．各冷鉱泉には稀少金属が多く含まれており，またその成分にも差があり鉱泉毎に特徴を持っている．これら冷鉱泉に関する地球化学的研究は数多く行われてきているが，生物学的研究は行われていないのが現状である．今回，各冷鉱泉に見られる茶褐色の沈殿物に関し生物学的に解析を試みた．沈殿物の電子顕微鏡（SEM）観察によると，その大部分は無機質で出来ており，核になるものの周囲に細かな沈殿物が付着して成長し，大きな沈殿物を形成している様に観察された．沈殿物の中には時折，細菌と思われる生命体も観察された．これらの沈殿物を含む鉱泉水を鉄酸化細菌用培地にて培養を試みたところ，わずかではあるが細菌の増殖が見られ，数年にわた

り同培地での培養が継続されている．この細菌は非常に増殖が悪い等の理由から種の同定は困難であったが，鉄を唯一のエネルギー源として増殖し，短桿菌という形態からすると *Acidithiobacillus ferrooxidans* と類似した細菌と考えられる．また，細菌数は非常に少ないものの，細菌が生育している培地の沈殿物は試験管のガラス壁に強固に付着しており，さらに光学顕微鏡観察により，沈殿物の結晶間に糸状の構造物が存在することが確認された．また，その沈殿物を形成している結晶の大きさも細菌が関与していない沈殿物と比べ大きかった．つまり，生物が関与せずに無機的に沈殿物が形成されるよりも，細菌が関与した場合の方がより強固な沈殿物が形成されることがわかった．沈殿物形成は無機的に行われるばかりでなく，微生物が関与して形成されることが実験によって示唆された．

キーワード：細菌，沈殿物，二酸化炭素分圧の高い冷鉱泉，プリモーリエ，糸状構造物，正８面体結晶

Abstract

The Tiga forest of Primorye is located in the Vladivostok and Khabarovsk regions of the Far East Russia. There are more than 100 springs with either Ca-$HCO_3$ or Na-$HCO_3$-type waters, and these contain high levels of $PCO_2$, ferrous ion and a variety of rare elements. Electron microscopic observation of deposits from these springs revealed that they were mostly formed by inorganic matter, and that they had a core of inorganic or organic material. Occasionally, bacterial organisms were found in the deposits. Cultivation of these spring waters with deposits showed a few bacterial growth in inorganic media with ferrous ion. According to these bacterial cultivated conditions using ferrous ion for their growth and their short-rod forms, these bacteria resembled *Acidithiobacillus ferooxidans,* but could not be identified precisely because of their difficult cultivation. After a few months' cultivation, deposits were formed with strongly adhered to the glass tube wall ; the deposits were difficult to dislodge with gentle tapping. The presence of web-like structures with octahedrons crystals in the cultivated deposits may explain the durability of the deposits ; these networks were not present in the control medium. These findings show that the formation of deposits in spring is strongly related to the presence of microorganisms such as bacteria.

Key words : bacteria, deposits, high $PCO_2$ spring, Primorye, string-like structure, octahedrons crystals

## 1. Introduction

The Tiga forest of Primorye is 250,000 km$^2$ in area, located in the Vladivostok and Khabarovsk regions in Far East Russia. This area is well-known for its abundant deposits of rare elements. More than 100 springs are located in the Primorye Area (Chudaev 1998–1999). Most of the springs are cold, and only 2 springs located near the Japan Sea side, can be called "hot springs". These springs have typically a high $PCO_2$ (up to 2.6 atm) and a high concentration of $Ca^{2+}$ and $HCO_3^-$, and are also important for $Na^+$, $Mg^{2+}$ or $K^+$ (Shand *et al.* 1995, Chudaeva *et al.* 1999, Chudaev *et al.* 2001). In addition, each spring contains a variety of rare elements (Shand *et al.* 1995, Chudaeva *et al.* 1995 and 1999, Chudaev *et al.* 2001). Fe ions are also high (Shand *et al.* 1995, Chudaeva *et al.* 1999, Chudaev *et al.* 2001), and most of these springs are orange due to ferrous deposits that had been oxidized from $Fe^{2+}$ to $Fe^{3+}$.

The chemical composition of the water of the high $PCO_2$ Schmakovka Spring, near Lake Khanka, was investigated in the 19th century. This work was performed by the Far East

Geological Survey, the Central Institute of Health and Physiology, and the Primorye Territorial Geological Survey. Most of the data from these projects were not published and are kept in the archives of the Far East Geological Survey and Primorye Territorial Geological Survey. After these studies, the springs were reevaluated for their use in energy production ; then, the heat sources of the Chistovodnoe Area and the possibility of mine deposits in the Lastochika and Gornovodnoe Areas were researched (Ushakin ed. 1969). Recently, Chudaeva and her team investigated the chemical composition of these springs, particularly those near Vladivostok City, and published the book, *Mineral Waters of Primorye* (Chudaeva *et al.* 1999). Their subsequent researches were continued, focusing on the springs of Primorye, and new geochemical data were presented (Chudaev *et al.* 2001). Most of these data were published in Russian, but some of the findings concerning the high $PCO_2$ spring water in Primorye have been presented in Japanese (Sugimori 2003).

These springs are classified as either Ca-$HCO_3$ or Na-$HCO_3$ springs. The chemical composition of these springs is characterized by the frequent presence of high concentrations of rare elements, especially Cu, Ga, and Ge, and by a high $PCO_2$ (Shand *et al.* 1995, Chudaeva *et al.* 1999, Chudaev *et al.* 2001, Sugimori 2003). In addition, the concentration of Fe is also high, resulting in red deposits. Although a great deal of geochemical and geological analyse of these springs have been done only a few studies have been reported on the microbiological properties. The purpose of the present study is to investigate the biological properties for these high $PCO_2$ springs.

## 2. Sampling field and methods

### 2.1 Sampling field

The fields of this study were in the Primorye Area, which is north of Vladivostok City, in the Far East Russia. Ten high $PCO_2$ spring waters with red deposits were sampled for bacteriological analysis in Medveji, Abdeevsky, Nerobinsky, Bolshoi Kluch, Sodovy, Narzany, Lenino-2, Luzky (2 samples) and Gornovodnoe. The pH and temperature of these springs were measured in the field with a handy pH meter (YOKOKAWA pH81) (Sugimori, 2003).

### 2.2 Electron microscopic observation of deposits in springs

Scanning electron microscopy (SEM, HITACHI ; S450) was used to observe deposits in water samples. Deposits were washed with 0.1M PBS and fixed with 2.5% glutalaldehyde (in 0.1M PBS). The fixed sample was placed on the thin cover glass (slide) coated with 0.2% poly-L-lysine, and viewed after staining with 1% osmic acid, and conductive staining with 2% tannic acid and 1% osmic acid.

### 2.3 Biological analyse of deposits

The deposits were cultured in 9K Medium, containing $K_2HPO_4$ : 0.5 g, $(NH_4)_2SO_4$ : 3.0 g, KCl : 0.1 g, $MgSO_4 \cdot 7H_2O$ : 0.5 g, $FeSO_4 \cdot 7H_2O$ : 50.0 g, $Ca(NO_3)_2$ : 10.0 mg, 10N $H_2SO_4$ : 1.0 m*l*, and distilled water : 1.0 liter. The pH of this medium was adjusted to 3.0, however it was actually little bit higher than 3.0, but no higher than 4.0. The reason for this is iron oxidizing bacteria can not be cultured in a medium over pH 4. After sterilization, 10m*l* of this medium was placed in each sterilized tube. Samples from these springs were inoculated into each tube with the medium, to a volume of 10%, and incubated at 30℃.

After several weeks culture, bacterial growths and the characteristics of deposits were observed by using a light microscope (OLYMPUS BX50).

## 3. Results and Discussion

Ten springs containing deposits were sampled in July and August 1999. The spring deposits were all reddish-brown. The pH and temperature of these springs are shown in Table 1 (Sugimori, 2003). These spring waters were cold and the pH was neutral to acidic. The other data regarding DO, $HCO_3$, EC, and chemical components are described in Shand *et al.* (1995), Chudaeva *et al.* (1999 in Russsian), Chudaev *et al.* (2001) and Sugimori (2003, in Japanese).

Electron microscopic images of the spring deposits are shown in Photos 1-1 to 1-5. Photo 1-1 confirms that the deposits were almost entirely formed of inorganic matter. Sometimes, the deposits exhibited linearity, as shown in Photo 1-2. Photo 1-2 shows inorganic matter adhering in a regular pattern to the surface of other lining materials. Rod shaped bacteria were found in the inorganic deposits, as shown in Photos 1-3 and 1-4. A bacterium Spirochete was also found in the deposits (Photo 1-5). The genus name of spices may be *Leptospira* with its typical shape of spiral, which is commonly found in natural sources contaminated with animal feces. Previous reports suggested that biological processes were responsible for the growth of these spring deposits (Konhauser *et al.* 1999, Sugimori *et al.* 1991).

After cultivating these spring waters with deposits at 30℃, small numbers of bacteria

Table 1 Temperature and pH of the high $PCO_2$ spring waters in the South Primorye, Far East Russia (1999 samples, Sugimori 2003)

| Locality | Temperature (℃) | pH |
|---|---|---|
| SHIMAKOVKA Group | | |
| 1. Medveji | 11.4 | 6.64 |
| 2. Abdeevsky | 10.1 | 6.50 |
| SHETUKHINSKAYA Group | | |
| 3. Nerobinsky | 7.4 | 5.72 |
| 4. Bolshoi Kluch | 8.0 | 6.53 |
| SAMARUKA Group | | |
| 5. Sodovy | 8.2 | 5.64 |
| LENINSKOE Group | | |
| 6. Narzany | 5.6 | 6.83 |
| 7. Lenino-2 | 8.8 | 4.42 |
| CHUGUEVKA Group | | |
| 8. Luzky | 11.8 | 6.62 |
| (borehole) | 16.5 | 6.62 |
| GORNOVODNOE Group | | |
| 9. Gornovodnoe | 9.3 | 6.77 |

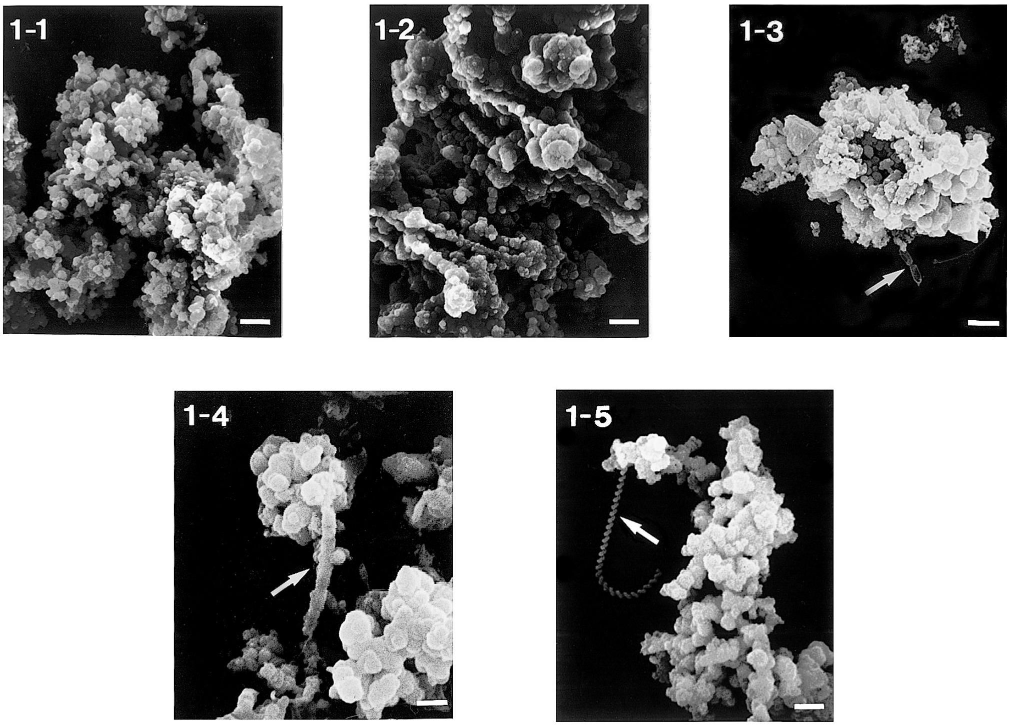



Photo 1　Electron microscopic images of spring deposits from Primorye, Russia
1-1　Sodovy Spring deposits (Bar＝2$\mu$m)
1-2　Bolshoi Kluch Spring deposits (Bar＝2$\mu$m)
1-3　Sodovy Spring deposits (Bar＝2$\mu$m)
1-4　Narzany Spring deposits (Bar＝1$\mu$m)
1-5　Bolshoi Kluch Spring deposits (Bar＝1$\mu$m)

were found in the culturees as showing an arrow (Photo 3-3). Bacteria capable of growing in inorganic medium by using ferrous ion for energy were found in all spring water samples from Primorye, but only one or two short-rod cells were found in about 10 fields under light microscopic observation. These bacteria were cultured for 7 additional years, with 3 or 4 inoculations a year, under inorganic medium conditions. These bacteria were grown in the medium with ferrous ion for one of the energy source for growth. According from these fact, the bacterium seems to be *Acidithiobacillus ferooxidans*, but not yet be identified precisely, because of the difficult cultivation. When the culture tubes were observed after a few months cultivation, cultured deposits were fixed on the glass tube wall, as shown in Photos 2-1 and 2-2. These deposits strongly adhered to the glass tube wall and maintained intact after gently shaking and tapping the test tube. However, the control tube deposits (uncultured medium, without bacteria) readily disintegrated after gentle shaking or tapping, as shown in Photo 2-3. After one or two months' cultivation, light microscopic observation show that crystals were found in the cultivated medium and in control medium (Photo 3). Crystals from cultured and control tubes were right octahedrons, and the crystals in control tube (Photo 3-2) were usually smaller than the crystals in cultured tube (Photo 3-1). Thin string-like structures

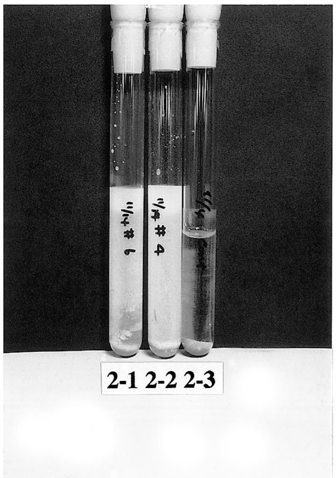
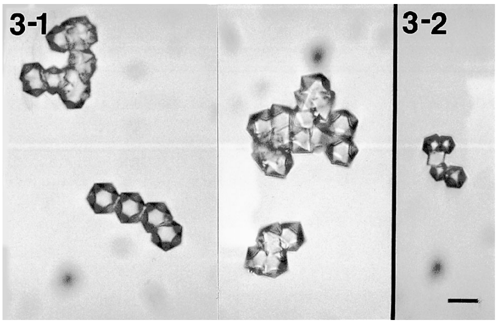
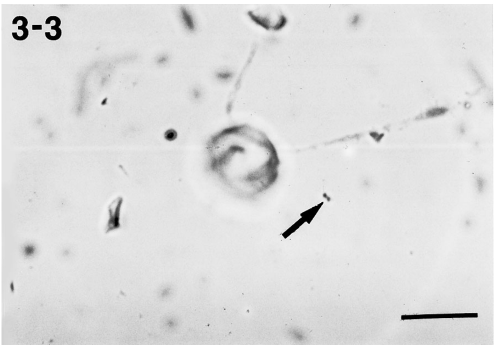



Photo 2 Cultured samples with deposits from Primorye, Russia and uncultured sample with deposits in test tubes
2-1 and 2 -2 : cultured samples, 2-3 : uncultured sample



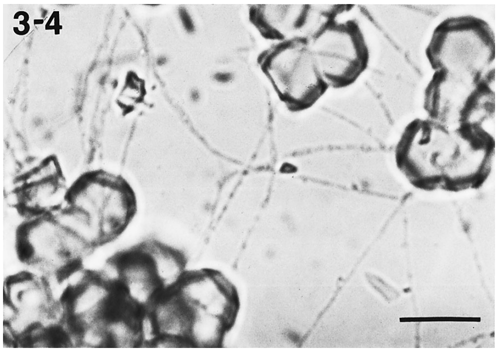




Photo 3 Light microscopic observation of crystals in cultured and uncultured samples
3-1 Crystals formed from a cultured sample (×400)
3-2 Crystals formed from an uncultured sample (×400)
3-3 Thin filaments and bacteria from a cultured sample (×1000)
3-4 Spider web structure linking octahedral crystals from a cultured sample (×1000)
(Bar＝10 $\mu$m)

were observed in cultivated deposits (Photos 3-3 and 3-4). These structures resembled a spider web, with a network among crystals, and they may be formed by bacterial activity. These networks were not observed in the control medium. These may explain why more resilient deposits were formed in the cultured medium. We can summarized the findings in previous papers about the relationships between microorganisms and deposits, as follows ; 1. Many kinds of microorganisms were found in the spring deposits, consisted algae mainly of found in the center of deposits and formed the deposits (Sugimori *et al.* 1991). 2. Some bacteria, cyanobacteria, were found in the silica sinter by electron microscopic observation, and their cytoplasms were replaced by silica, presumably by lysed process *in-situ* (Konhauser *et al.* 1999). And one of the cyanobacteria, *Calothrix* sp., were isolated from the surface of silica sinter, and cultured (Konhauser *et al.* 1999). So, the existence of cyanobacteria was so important for the silicification at the hot spring in Iceland (Konhauser *et al.* 1999). 3. It was revealed that iron and silica were accumulated inside of bacteria as biomineralization at Yellowstone National Park (Ferris *et al.* 1986).

These reports and our findings show that the formation of deposits in spring is strongly correlated with the presence of microorganisms.

## References

Chudaeva, V.A., Lutsenko, T.N., Chudaev, O.V., Chelnokov, A.N., Edmunds, W.M. and Shand, P. (1995) : Thermal waters of Primorye region, Eastern Russia. Proceedings of Water Rock Interaction 8th (by Kharaka, Y. and Chudaev O.V. ed.), 375–378, Balkema, Rotterdam.

Чудаева (Chudaeva), B.A., Чудаев, O.B., Челноков, A.H., Здмундс, У.M., Шана, П. (1999) : Минеральиые Воды Приморья (Mineral Waters of Primorye). 172 p, Далънаука・Владивосток.

Chudaev, O.V. (1998–1999) : Mineral waters of Primorye. Printed in Kongju University of South Korea in 1998–1999 (short report with 4 pages).

Chudaev, O.V., Chudaeva, V.A., Sugimori, K., Nagao, K., Takano, B., Matsuo, M., Kuno, A. and Kusakabe, M. (2001) : New geochemical data of the high $PCO_2$ waters of Primorye (Far East Russia). Proceedings of Water Rock Interaction 10th (by Cidu, R. ed.), Vol. 1, 473–476, Balkema, Lissa.

Ferris, F.G., Beveridge, T.J. and Fyfe, W.S. (1986) : Iron-silica crystallite nucleation by bacteria in a geothermal sediment. Nature, **320**, 609–611.

Konhauser, K.O., phonenix, V.R., Bottrell, S.H., Adams, D.G. and Head, I.M. (1999) : Microbial—silica interaction in modern hot spring sinter. P. 263–266, Gheochemistry of the Earth's Surface (Armannsson ed.), A.A. Balkema, Rotterdam.

Shand, P., Edmunds, W.M., Chudaeva, V.A., Lutsenko, T.N., Chudaev, O.V. and Chelnokov, A. N. (1995) : High $PCO_2$ cold springs of the Primorye region, Eastern Russia. Proceedings of Water Rock Interaction 8th (by Kharaka, Y. and Chudaev O.V. ed.), 393–396, Balkema, Rotterdam.

Sugimori, K., Mifune, M. and Yuhara, K. (1991) : Biological studies of spring deposits situated in Kuroiwa Spring, Kyushu. J. Balneol. Soc. Jap. **41** (3), 169–177.

Sugimori, K. (2003) : High $PCO_2$ spring water in the Primorye, Far East Russia (in Japanese) J. Balneol. Soc. Jap. **53** (2), 48–60.

Ushakin, E.P. ed. (1969) : Приморский край (Primorye Teritory) Гидрогеология СССР (Hydrogeology of USSR, vol. XXV), 520 p, M. Недра, Москва (Moscow)