Relation between Bacteria and Deposits found in the High
PCO₂ Spring Waters of Primorye, Far East Russia

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Abstract

The relation between bacteria and deposits found in the high 
PCO₂ Spring Waters of Primorye, Far East Russia 
was studied. The deposits were composed of 
calcium carbonate and iron oxides. The bacteria 
were observed using SEM. The bacteria were 
found to be associated with the deposits. 
The results suggest that bacteria play an 
important role in the formation of the deposits.

Keywords: Bacteria, Deposits, Primorye, Pacific Institute of Geography, Russian Academy of Sciences

Introduction

The Spring Waters of Primorye, Far East Russia, 
are known for their high PCO₂ content. 
Previous studies have shown that 
the deposits formed in these waters 
are composed of calcium carbonate 
and iron oxides. 

Methods

Samples were collected from the Spring Waters of Primorye. 
The deposits were analyzed using X-ray diffraction. 
The bacteria were observed using SEM.

Results

The deposits were composed of calcium carbonate 
and iron oxides. 
The bacteria were associated with the deposits.

Discussion

The results suggest that bacteria play an important role in 
the formation of the deposits. 
The calcium carbonate and iron oxides 
are likely to be precipitated by bacteria.

Conclusion

The relation between bacteria and deposits found in the high 
PCO₂ Spring Waters of Primorye, Far East Russia 
was studied. 
The bacteria were found to be associated with the deposits. 
The results suggest that bacteria play an important role in 
the formation of the deposits.

Acknowledgments

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Russian Academy of Sciences.

References

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 $PCO_3$, 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 ferrooxidans, 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 $PCO_3$ 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 $PCO_3$ (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 $PCO_3$, 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. +303). 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, **-177).

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 analysis 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% gluteraldehyde (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, $(NH_4)SO_4$ : 3.0 g, KCl : 0.1 g, MgSO$_4$ · 7H$_2$O : 0.5 g, FeSO$_4$ · 7H$_2$O : 50.0 g, Ca(NO$_3$)$_2$ : 10.0 mg, 10N H$_2$SO$_4$ : 1.0 ml, 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, 10 ml 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°C.

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, HCO₃, EC, and chemical components are described in Shand et al. (1995), Chudaeva et al. (1999 in Russian), 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 spics 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°C, small numbers of bacteria

<table>
<thead>
<tr>
<th>Locality</th>
<th>Temperature (°C)</th>
<th>pH</th>
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<tbody>
<tr>
<td>SHIMAKOVKA Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Medveji</td>
<td>11.4</td>
<td>6.64</td>
</tr>
<tr>
<td>2. Abdeevsky</td>
<td>10.1</td>
<td>6.50</td>
</tr>
<tr>
<td>SHETUKHINSKAYA Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Nerobinsky</td>
<td>7.4</td>
<td>5.72</td>
</tr>
<tr>
<td>4. Bolshoi Kluch</td>
<td>8.0</td>
<td>6.53</td>
</tr>
<tr>
<td>SAMARUKA Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Sodovy</td>
<td>8.2</td>
<td>5.64</td>
</tr>
<tr>
<td>LENINSKOE Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Narzany</td>
<td>5.6</td>
<td>6.83</td>
</tr>
<tr>
<td>7. Lenino-2</td>
<td>8.8</td>
<td>4.42</td>
</tr>
<tr>
<td>CHUGUEVKA Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Luzky</td>
<td>11.8</td>
<td>6.62</td>
</tr>
<tr>
<td>(borehole)</td>
<td>16.5</td>
<td>6.62</td>
</tr>
<tr>
<td>GORNOSVODNOE Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Gornovodnnoe</td>
<td>9.3</td>
<td>6.77</td>
</tr>
</tbody>
</table>
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 ferrooxidans*, 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
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 cytoplasm 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 impor-
tant for the silicification at the hot spring in Iceland (Konhauser et al. 1999). 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.

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