Biological Studies of Spring Deposits Situated in Kuroiwa Spring, Kyushu

Kenji SUGIMORI*, Masaaki MIFUNE**
Kozo YUHARA***

*Department of Biology, Toho University School of Medicine
**Formerly Misasa Hospital, Okayama University Medical Shool
***Community Technology Reseach Co., Ltd.

Received 10 Jan. 1991, Accepted 31 Jan. 1991

Introduction

Hot spring deposits are formed from dissolved matter in spring waters undergoing physical and chemical changes in high-pressure or hot water erupting from underground. The first report of chemical analysis of hot spring deposits was published by Ohashi R. (1920) on radioactive deposits at Tamagawa Hot Springs, Akita Prefecture in Japan[1]. After that, many papers have been published on hot spring deposits[2~7], and deposits were classified according to chemical components of the waters; the categories sulfur, sulfides, hydrous ferric oxide, hydrous silica and carbonates[2].

Until now, hot spring deposits have been studied only chemically. But at this time we are focusing on the formation of the deposits, which were observed with the naked eyes and also involves the participation of microorganisms. Therefore we undertook a biological study, that is, light and electron microscopic observations and biological characteristics of microorganisms of Kuroiwa Spring, Fukuoka Prefecture. The many kinds of microorganisms observed and isolated are reported herein.

Materials and Methods

1. Samples

The spring deposit samples used in this study were collected from Kuroiwa Spring (No.1) in Sedaka-Cho, Fukuoka Prefecture on May 5th, 1988 (Fig. 1). The deposits were located at the bottom of a small puddle. The deposits and spring waters were collected in polycarbonate bottles (250ml). The temperature and pH value of the water were measured at that time.

2. Light microscopic observation of deposits

The samples were observed under light microscopy, and representative photographs were taken. The methods of observation were as follows: a) fresh samples were used for observation by phase-contrast microscope; b) Gram's method was used for the preparation of fresh samples;

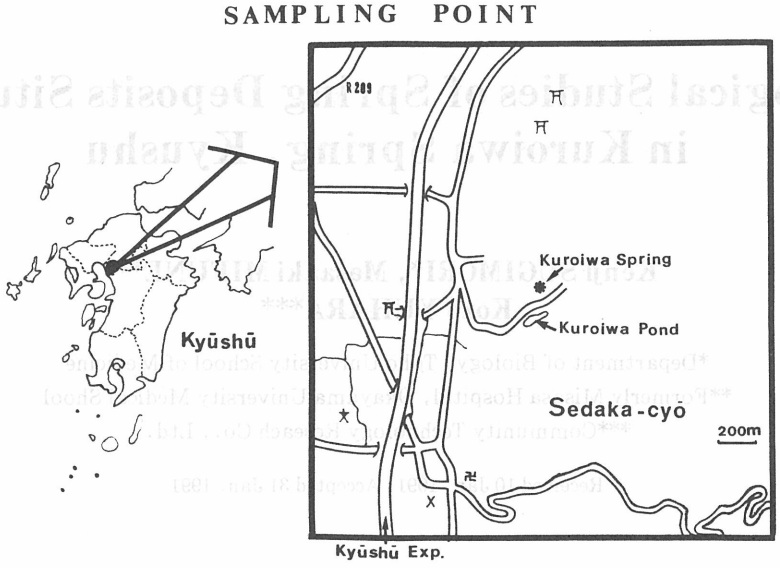

Fig. 1 The location of Kuroiwa Spring in Fukuoka Prefecture

c) samples that were fixed with formalin and no staining, were observed by direct observation method. Identification of microorganisms was performed using the following textbooks: "Illustration of the fresh water plankton of Japan[8]", "Illustration of the fresh water algae in Japan[9]", "Encyclopaedia zoollogica illustrated in colours 4[10]" and "Photomicrographs of the fresh-water algae[11]".

3. Electron microscopic observation

First, samples deposits were washed with 0.1M PBS buffer, and fixed by 2.5% glutal aldehyde (in 0.1M PBS buffer). The thin cover glass was coated with 0.2% poly-L-lysine, and the fixed sample placed on the coated glass (slide), and used for after staining (1% osmic acid and conductive staining method 2% tanic acid and 1% osmic acid). A scanning electron microscope (SEM) was used for observation of the samples.

4. Isolation and identification of bacteria from deposits

a) Medium

Nutrient broth (Nissui), Nutrient agar (Nissui) and Thiobacillus identification medium[12] were used for isolation of bacteria living in the spring deposits. The components of Thiobacillus identification medium were as follows: $MgSO_4 \cdot 7H_2O$; 0.20g, K_2HPO_4; 1.00g, $FeSO_4 \cdot 7H_2O$; 0.05g, $CaCl_2$; 0.02g, NH_4Cl; 1.00g, $MnCl_2 \cdot 4H_2O$; 0.002g, $Na_2MoO_4 \cdot 2H_2O$; 0.001g, $Na_2S_2O_3 \cdot 5H_2O$; 7.00g, purified agar (Oxoid) (if neccessary); 12g in 1000ml of distilled water (The pH of medium will be 7.4 after mixture).

b) Isolation

Spring deposit/spring water suspension was added to each medium, at a volume of 10%, and incubated at 30℃ for 1 week. After that, the turbidity of the medium was confirmed, and medium was streaked on a medium plate, as mentioned above.

c) Characterization of bacteria

Bacteria colonies were observed for color, form and diameter. Gram stained bacteria were observed by light microscope, and the following tests were carried out on heterotrophic bacteria: catalase test, oxidase test, motility test, growth condition test, oxidation or fermentation of glucose test, acid from glucose test, inositol and saccharose test, growth in 6% NaCl or without NaCl test, lysine decarboxylase test, ornithine decarboxylase test, gelatine hydrolysis test and starch hydrolysis test. Genetic names were decided. Autotrophic bacteria were grown in Thiobacillus identification medium, and identified as *Thiobacillus spp.*.

Results and discussion

White-color deposits were observed at the bottom of a small puddle of Kuroiwa Spring (Photo 1). The chemical components of the spring waters are presented in Table 1. It was recognized that the spring waters belonged to Ca-SO_4 type simple cold waters. The temperature and pH value of the spring waters were 21.7℃ and 7.2, respectively.

Table 1 Chemical components of the Kuroiwa Spring (No.1)

Chemical components	(mg/kg)
K	0.9
Na	4.2
Ca	52.4
Mg	12.5
Fe	tr
Mn	0.30
Cl	4.6
SO_4	168.0
HCO_3	17.0

tr: trace

In both light microscopic and electron microscopic observations, many kinds of microorganisms were observed in the deposits.

In the protozoa group, *Trinema sp.*, *Euglypha sp.* and *Paramecium sp.* were identified from their morphological characteristics (Photo 2). Especially, *Paramecium sp.* which showed active movement with a peritrical flagella was observed in the deposits, using the phase-contrast microscopic method (Photo 2-3).

Photo 1 Sampring point of Kuroiwa Spring
(near flow out point)

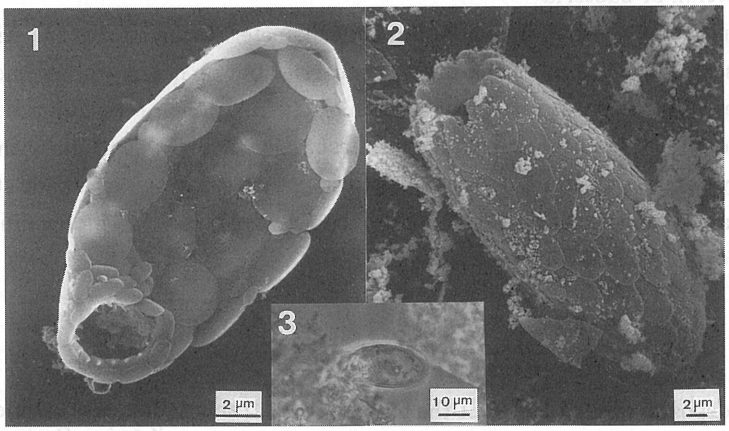

Photo 2 Photographs of protozoa detected from the deposits
(1 and 2 : electron microscopic observation, 3 : light microscopic observation of phase contrast method)
1) Trinema sp.
2) Euglypha sp.
3) Paramecium sp.

In the blue-green algae group, *Chroococcus minutus var. minutus*, *Phormidium lepideum var. amorphum*, *Scytonema sp.*, *Calothrix braunii* and *Anabaena sp.* were identified from their remarkably morphological characteristics (Photo 3). In *Scytonema sp.*, false branches arose in unilateral pairs (Photo3-5-e). In *Calothrix braunii*, trichomes were tapered from basal heterocyst to a fine point, abruptly ending in the others (Photo 3-3). Among these algae, *Scytonema sp.* and *Calothrix braunii* occupied the majority of the deposits. *Anabena sp.* was identified by their remarkable heterocyst, akinete and tricome cell (Photos 3-1 and 2). On the other hand, by the use of tram staining, heterocysts and akinetes were stained to gram positive, and vegetative cells were stained to gram negative, as shown in Photo 4.

In the diatomaceae group, *Navicula cuspidata*, *Nitzschia capitalla*, *Pinnularia microstauron*, *Eunotia arcus*, *Eunotia sp.* were identified by their morphological characteristics under electron microscopic observation (Photo 5). Rostrum were slightly inflated at the apex in *N. capitalla* (Photo 5-1), and this diatoma was reported to be rarely identifed in Japan. So, it is considered very significant that *N. capitalla* was found in the springs in Kyusyu.

In the bacteria group, short rod and rod shape were observed in the deposits by electron microscopic observation. Characterization of the bacteria, revealed that 3 different types of bacteria were isolated from the deposits (Table 2). As for, short rod bacteria, these strains grew in the autotorophic medium containing thiosulfate as the only energy source. Therfore, the strain was determined to be *Thiobacillus spp.*. As for the rod-shape bacteria, remaining 2 strains showed heterotrophic growth. According to the results of characterization of heterotrophic bacteria, these strains were determined to be *Aeromonas spp.* and *Alcarigenes spp.*.

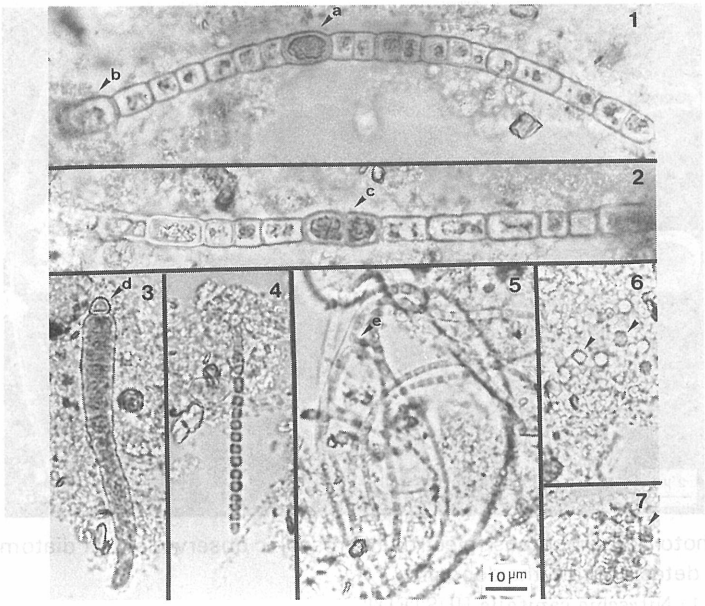

Photo 3 Photographs (light microscopic observation) of blue-green algae detected from the deposits
1 and 2) Anabaena sp. (a and c; heterocyst, b; akinete)
3) Calothrix braunii BORNET et FLAHAULT (d; heterocyst)
4) Pholmidium lepideum GEITLER var. amorpham EMOTO et HIROSE
5) Scytonema sp. (e; false branching)
6 and 7) Chroococcus minutus (KÜTZING) NAEGELI var. minutus

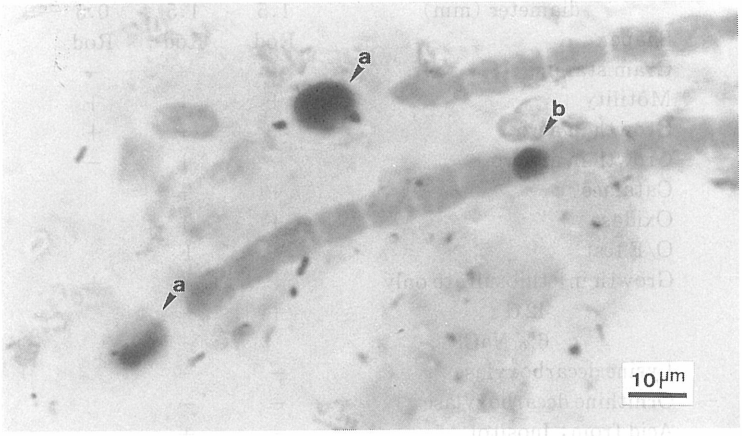

Photo 4 Gram stained cells of Anabaena sp.
(a; akinate, b; heterocyst)

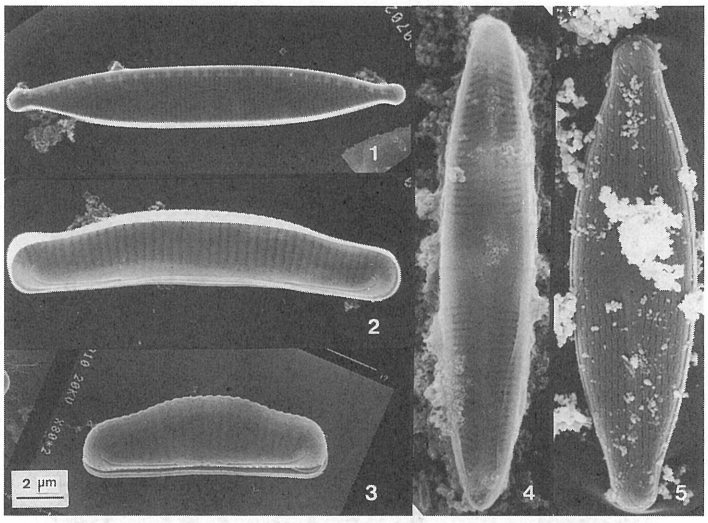

Photo 5 Photographs (electron microscopic observation) of diatoma detected from the deposits
1) Nitzschia capitalla HUSTEDT
2) Eunotia arcus EHRENBERG
3) Eunotia sp.
4) Pinnularia microstauron (EHRENBERG) CLEVE
5) Naviculla cuspidata KÜTZING

Table 2 Characteristics of 3 kind of bacteria isolated from the deposits

	Isolated strains		
	88－1	88－2	88－3
Colony: color	W	W	W
shape	S	R	S
diameter (mm)	1.5	1.5	0.5
Shape	Rod	Rod	Rod
Gram staining	－	－	－
Motility	＋	＋	＋
Growth in aero.	＋	＋	＋
Growth in anaero.	－	＋	－
Catalase	＋	＋	
Oxidase	＋	＋	
O/F test	－	F	
Growth in: thiosulfate only			＋
42℃	＋		
6% NaCl		－	
Lysine decarboxylase	－	－	
Ornithine decarboxylase	－	－	
Acid from: Inositol	－	＋	
Saccharose	－	＋	
Gelatine hydrolysis		＋	
Starch hydrolysis		＋	

W: White, S: Smooth, R: Rough

In all, 3 different types of protozoa, 5 different types of blue-green algae, 5 different types of diatoma and 3 different types of bacteria were observed and isolated in the soft type deposits of Kuroiwa Spring (Table 3). It seems that the deposits are mainly composed of those microorganisms, and especially blue-green algae was at the most important constituent of the deposits. This opinion is based on the observations under electron microscope and light microscope. The three photographs in Photo 6 show that many kinds of blue-green algae clung together, and materials seeming to be nonorganic existed on or around the algae. Thus, it seems that the deposits in this study visible with the naked eye were made up of blue-green algae growth. In detail, small deposits that could not be seen with the naked eye were made up of "chemical deposits", which, when setting on the algae, grew bigger with more and more chemical deposits. After that, the deposit became a "house-like composer" for protozoa, diatoma and bacteria, thus the small free-living organisms

Table 3 Species name of protozoa, blue-green algae, diatoma and bacteria detected from the deposits

Protozoa	Trinema sp.
	Euglypha sp.
	Paramecium sp.
Blue-green algae	Chroococcus minutus (KÜTZING) NAEGELI var. minutus
	Calothrix braunii BORNET et FLAHAULT
	Phormideum GEITLER var. amorphum EMOTO et HIROSE
	Anabaena sp.
	Scytonema sp.
Diatoma	Naviculla cuspidata KÜTZING
	Nitzschia capitalla HUSTEDT
	Pinnularia microstauron (EHRENBERG) CLEVE
	Eunotia arcus EHRENBERG
	Eunotia sp.
Bacteria	Alcarigenes sp.
	Aeromonas sp.
	Thiobacillus sp.

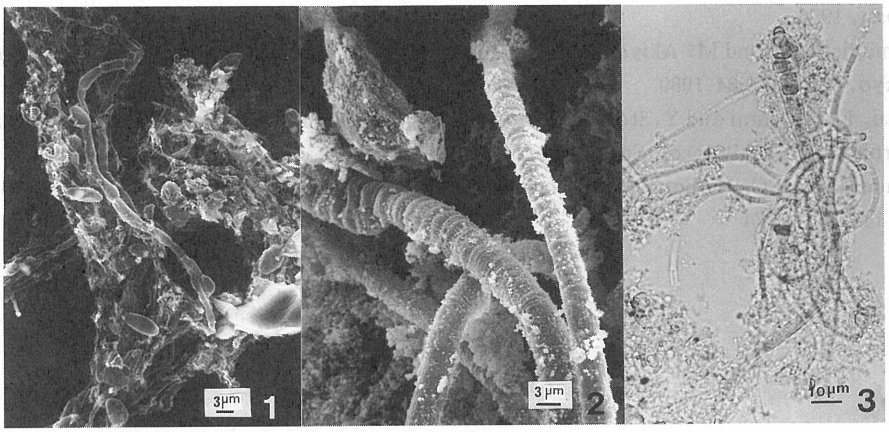

Photo 6 Photographs (1 and 2 : electron microscopic observation, 3 : light microscopic observation) of deposits: many kinds of blue-green algae were cling together

were thought to live in it (Photo 6). Although a report by Yamazaki Y. (1973), mentions some leaves, seeds and spiral shells being detected in the hot spring deposits in Shiobara Area with the substance microscopie[13]. This paper was the first to show that many kinds of microorganisms actually thrive in hot spring deposits.

Acknowledgements

We are grateful to Dr. Tsugio Shiroya, Dr. Shinnosuke Takayanagi and Dr. Kazuyuki Oka of department of biology, Toho University school of medicine for useful comments.

References

1) Ohashi, R.: Note on the plumbiferous Barytes from Shibukuro, prefecture of Akita, Japan., Mineral Mag, J. Mineral Soc., 19: 73-76, 1920
2) Ichikuni, M.: Hot spring deposits., J. Balneol.Soc. Japan, 24: 49-54, 1973
3) Saito, N.: Radioactive sinter deposits., J. Balneol. Soc. Japan, 27: 31-35, 1976
4) Yoshida, T., K. Yuhara, Y. Nakae and T. Noda: Hot-water and precipitates in the hot pool "Chinoike-jigoku", Beppu geothermal area, Kyushu, Japan., J. Balneol. Soc. Japan, 29: 10-18, 1978
5) Yokoyama, T. and T. Tarutani: Silica in ferruginous sinters., J. Balneol. Soc. Japan., 30: 75-83, 1979
6) Yuhara, K., S. Ehara, T. Noda and S. Nakao: Changes in depth, temperature, chemical composition, precipitate, heat discharge and water discharge of the hot pool "Chinoike-jigoku" Beppu, Kyushu, Japan., J. Balneol. Soc. Japan, 32: 153-163, 1982
7) Sakai, S. and R. Suzuki: Chemical composition changes of hot spring waters and deposites in transportation by pipelines at Asamakogen and Ikaho Hot Springs, Gunma Prefecture., J. Balneol. Soc. Japan, 36: 115-124, 1986
8) Mizuno, T.: Illustration of the fresh water plankton in Japan., Hoikusya, Osaka, Japan, 1983
9) Hirose, Y. and T. Yamagishi: Illustration of the fresh water algae in Japan., Uchidarokakuho, Tokyo, Japan, 1977
10) Okada, K. and T. Uchida: Encyclopaedia zoologica illustrated in colours 4., Hokyryukan, Tokyo, Japan, 1976
11) Yamagishi, T. and M. Akiyama: Photomicrographs of the fresh-water algae., Uchidarokakuho, Tokyo, Japan, 1984-1989
12) Goto, E., H. Imai and Y. Ito: Studies on the sulphur bacteria inhabited in the spa. 1. Culture and morphology., J. Balneol. Soc. Japan, 16: 144-153, 1966
13) Yamazaki, Y.: On the hot spring deposits near Shiobara, Tochigi prefecture., J. Balneol. Soc. Japan, 24: 148-157, 1974

福岡県黒岩湧水にて採取した沈澱物の生物学的検討

杉森賢司，御舩政明，湯原浩三

温泉沈澱物に関しては現在まで化学的なアプローチが主に行われてきたが，生物学的研究は行われていないのが現状である。しかし，その成因については生物の関与も大きいと考え，黒岩湧水の湧出口付近にみられた沈澱物について生物学的考察を行った。その結果，沈澱物中から細菌類（3種），藍藻類（5種），珪藻類（5種），原生動物（3種）が検出された。さらに沈澱物の電子顕微鏡観察により，その沈澱物は藍藻類がからみあってその中核をなしているのが観察され，検出された珪藻類，細菌類，原生動物はあたかもそこを宿にしているかのごとく生息していることがわかった。さらにそのまわりに無生物と思われる（いわゆる化学的な物質と考えられる）付着物が確認された。それが徐々に成長し大きくなって目に見える沈澱物になると考えられる。この様に，一見化学的に出来たと考えられがちな温泉沈澱物であっても，その成因には実際に生物が関与しており，これらのことから，沈澱物を考える上で生物学的ファクターは非常に重要であると考える。

Key words; Spring deposits
Blue-green algae
Bacteria
Diatoma
Protozoa