

Microbial Mat Boundaries between Chemolithotrophs and Phototrophs in Geothermal Hot Spring Effluents

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Abstract

Among the various microbial mats that develop in geothermal hot springs in solfataric fields, colorless sulfur-turf (ST) – macroscopic white bundles consisting of large sickle-shaped bacteria belonging to *Aquificales* and elemental sulfur particles – develops in a limited environment of geothermal effluent containing hydrogen sulfide with neutral pH and low in oxygen. Photosynthetic cyanobacterial mat (CY) often grow just downstream of chemolithotrophic ST, or they coexist with ST where the temperature is slightly lower. Knowledge of the environmental regimes of these microbial mats will lead to better understanding of the distribution of thermophilic microorganisms on the Earth and provide clues about evolutionary processes in the microbial ecosystems of the Precambrian era.

We studied the environmental parameters of the boundary zone and examined the distribution of these types of mats and measured the in situ growth rates of the microorganisms composing them. In situ examination revealed that temperature and Eh constrain the development of the microbial mats. At the boundary between ST and CY, temperature and Eh ranged between 51.1 °C and 63.2 °C and between –112 mV and –25 mV, respectively. These environmental parameters were not significantly different among Japanese, Yellowstone (North American), and Icelandic hot spring effluents with genetically similar thermal sulfur oxidizers. Sickle-shaped bacteria rarely coexist with cyanobacteria, although they can potentially grow in some CY environments. This suggests that the boundary between ST and CY might be partly determined by exclusive ecological competition.

Key words: boundary, chemolithotroph, cyanobacterial mat, hot spring, oxidation–reduction potential, phototroph, sulfur-turf, temperature gradient

Introduction

If geothermal hot spring effluent contains some amount of hydrogen sulfide and less than 1–2 mg/L dissolved oxygen, a conspicuously white feather-like microbial mat called sulfur-turf (ST) often develops in the effluent at temperatures above about 60 °C and with neutral pH, as was suggested by Maki (1991). There are relatively few descriptions of colorless sulfur bacterial mats or streamers in hydrogen sulfide-rich hot springs (Reysenbach et al. 1994, Skirnisdottir et al. 2000), although various photosynthetic bacterial mats in geothermal environments are well studied (Castenholtz 1976, Ferris et al. 1996, Ferris and Ward 1997, Hugenholtz et al. 1998, Ward et al. 1998, Ramsing et al. 2000). Previous studies have characterized the physiological and ecological features of the constituents of ST (Maki 1987a, 1987b, 1993), but the large sickle-shaped bacteria that predominate in ST remain uncultivated. Maki (1987b) showed that the microorganisms constituting ST chemolithotrophically oxidize sulfide to elemental sulfur, and then oxidize elemental sulfur to sulfate via thiosulfate. The 16S rRNA gene sequence from ST mats suggests that this large sickle-shaped bacterium belongs to the order *Aquificales*, which are deeply branching bacteria on the 16S rRNA phylogenetic tree (Yamamoto et al. 1998).

Hot spring effluent also provides habitats for various photosynthetic prokaryotes, including cyanobacteria and *Chloroflexus*, when light is available, (Castenholtz 1976, Ward et al. 1998, Hiraishi et al. 1999). If the temperature is less than about 60 °C, photosynthetic bacteria construct green, orange, olive-green, or purple microbial mats in the hot springs so far examined in Japan.

ST and photosynthetic bacterial mats often develop along a thermal gradient of the effluent of a geothermal hot spring. These microbial mats are usually in contact with each other, but the boundaries between them are distinct, in particular in the Japanese hot springs that have been examined (Fig. 1). Thus, a marked change in the trophic dynamics of microbial communities is noticeable along the temperature gradient of hot

spring effluents.

Microorganisms that obtain energy chemolithotrophically, such as *Aquificales*, are considered to have composed the first ecosystems on the early Earth, before the appearance of the photosynthetic bacteria, although what electron acceptor was available in the ancient atmosphere is still uncertain (see Vargas et al. 1998). Subsequently, the sustaining energy machinery of the ecosystem evolved from chemotroph to phototroph. In support of this, the 16S rRNA gene-based phylogeny shows that *Aquificales* and related bacterial strains that predominate in sulfur-rich hot springs are more deeply branching than the photosynthetic bacteria (Knoll 1999). It is quite likely that ST and other colorless mats in sulfide-rich hot springs preserve an ancient ecological feature (Reysenbach and Cady 2001). Thus, it is exciting to examine where and how ST develops and how it affects or is affected by the development of photosynthetic bacterial mats nearby.

Little is known about the environmental factors that determine the boundary between such colorless sulfur mats and photosynthetic bacterial mats. Thus, we examined the distribution pattern of ST microbial mats by measuring environmental parameters and estimating the in situ growth rate of the sickle-shaped bacteria in neutral hydrogen sulfide-rich hot spring effluent to reveal the mechanism by which the boundaries between microbial mats are determined. The field survey was extended to Yellowstone National Park in 1999 and 2000 to compare environmental constraints on the growth of genetically related thermal sulfur oxidizers.

Materials and Methods

Research Sites

We examined three geographically isolated hot springs located in Nagano prefecture, Japan: Nakabusa [lat 36°23'N, long 137°45'E, 1390 m above sea level (ASL)], Nakanoyu (lat 36°11'N, long 137°37'E, 1300 m ASL), and Yumata (lat 36°24'N, long

137°41'E, 1430 m ASL). Geothermal microbial mats have developed on the rocks at Nakanoyu and in the riverbed at Yumata. At Nakabusa, the effluent flows down the concrete wall of a dam, and the microbial mats have developed separately along small hot-water streams issuing from cracks in the dam (Sugiura et al. 2001). The field experiment in Yellowstone National Park was carried out in a pool near Fire Hole Lake (lat 44°32'N, long 110°47'W) and at Tangerine Mound and Bath Lake Vista of Mammoth Springs (lat 44°57'51"N, long 110°42'45"W).

In Situ Examination

Environmental parameters were examined by using the following digital meters: a digital thermometer with a probe 3 mm in diameter (DIGITAL HAND-THERMO, Sanyo Co. Ltd., Osaka, Japan) for measurement of temperature, a model CM-14P electrical conductivity (EC) meter with a 10-mm-diameter probe (DKK-TOA Co. Ltd., Tokyo, Japan), a model HM-14P pH meter with a 6-mm-diameter probe (DKK-TOA Co. Ltd. Tokyo, Japan) or a model HPH-110 pH meter with a 6-mm-diameter probe (DKK-TOA Co. Ltd. Tokyo, Japan), a model RM-14P oxidation–reduction potential (ORP) meter with a 4-mm-diameter probe (DKK-TOA Co. Ltd. Tokyo, Japan), and a model DO-14P dissolved oxygen concentration (DO) meter with a 3-mm-diameter probe (DKK-TOA Co. Ltd. Tokyo, Japan) or with a 12-mm-diameter probe (MODEL 58, YSI Co., Yellow Spings, Ohio, U.S.A.). The ORP was converted to Eh using a reference electrode. The dissolved sulfide concentration of the water was measured in situ with a simple tube kit for sulfide ion (No. 211, GASTEC Co. Ltd.). Measurements of dissolved hydrogen sulfide concentration taken with the kit were compared with spectrophotometric results (Cline 1969) obtained in 2000.

The employed probes were directly immersed in the water, and we measured environmental parameters just above the mats. However, at Nakabusa hot spring, to measure EC, pH, Eh, and DO, we carefully collected water from the effluent flowing

down the wall of the dam in a jug, and then took the measurements immediately.

Collected microbial mats were separated quickly into two subsamples for microscopic observation and for measurement of the absorption spectrum. Subsamples for microscopic observation were fixed immediately with 2% (v/v) formaldehyde neutralized with hexamethylenetetramine ((CH₂)₆N₄), and stored at room temperature until observation. Subsamples for the measurement of absorption spectra were stored in a refrigerator in the dark until measurement.

Chemical Analysis of Hot Spring Effluents

Water samples for chemical analysis were stored in 50-mL polyethylene bottles at -20 °C prior to analysis. NO₂⁻ and NO₃⁻ concentrations were determined by using ion chromatography (Dionex, Analyzer with Ion pack AS4A for the determination of anion, Sunny Valley, CA, U.S.A.). PO₄³⁻ and NH₄⁺ concentrations were measured by spectrophotometry according to the method of Strickland and Parsons (1972).

Microscopic and Spectroscopic Observations of Microbial Mats

A fraction of the fixed subsamples of microbial mats were air dried on glass slides, and stained with 4',6-diamidino-2-phenylindole (DAPI; 1 µg mL⁻¹ in distilled water filtered through a 0.22-µm-pore-size polycarbonate membrane filter; Nuclepore Corp., Pleasanton, CA, USA). The constituents of the microbial mats were observed by epifluorescence microscopy (BX50, OLYMPUS, Tokyo, Japan) with UV excitation.

Unfixed subsamples of microbial mats were directly suspended in distilled water, and the absorption spectra were measured between 600 nm and 900 nm with a spectrophotometer (model U-3200, Hitachi Co. Ltd., Tokyo, Japan).

Growth Experiment

To estimate sickle-shaped bacterial growth at the boundary between ST and

photosynthetic cyanobacterial mat (CY), an experiment was carried out at Nakabusa hot spring on 9, 22, and 30 October 1998. Glass slides, which had been previously sterilized in a dry oven at 120 °C for 1 h, were immersed in hot spring effluent. Paired glass slides were immersed for 6 h (6H) or 12 h (12H) in effluents where the temperature ranged from 50 °C to 60 °C. Collected glass slides were dried and stored in a refrigerator (< 4 °C) in the dark until counting. Microorganisms on the slides were stained with DAPI, and cells of total microorganisms, sickle-shaped bacteria, and cyanobacteria were counted on the basis of morphological distinctions observed under an epifluorescence microscope connected to a chilled 3-chip color CCD camera (HAMAMATSU Co. Ltd., Hamamatsu, Japan) and a color image analyzer (SP500F, Olympus Co. Ltd.). The growth rate constant (μ) was estimated from the number of attached cells at 6H and 12H. We estimated the attachment rate from the number of attached cells at 6H, although in situ growth might have occurred if the doubling time was as high as 6 h (see Results).

Results

General Description of the Hot Springs

Physical and chemical parameters of the effluent at the source of each hot spring are shown in Table 1. The water was hotter than 50 °C at all hot springs. Among the chemical parameters of hot spring effluent, the EC of the water differed among the three sites, ranging from 31.8 mS m⁻¹ (Nakabusa) to 183.7 mS m⁻¹ (Yumata). The pH was about neutral but differed somewhat among the three hot springs, ranging from 6.22 (Nakanoyu) to 8.44 (Nakabusa). The waters of the three hot springs contained sulfide with more than 2 mg L⁻¹, measured by a simple tube kit in situ, and the concentration of dissolved oxygen (DO) was less than 1.66 mg L⁻¹. The Eh of the water was less than 11 mV for all effluent sources. The hydrogen sulfide concentration determined by the

spectrophotometric method ranged from 0.07 to 9.37 mg L⁻¹ for the Nakabusa and Nakanoyu hot spring effluents, which was in nearly the same range as that measured previously by using the kit (Table 1). At Nakanoyu hot spring, the concentration of PO₄³⁻ in the effluent ranged from 0.28 mg L⁻¹ to 0.30 mg L⁻¹, that of NH₄⁺ ranged from 0.66 to 0.70 mg L⁻¹, and that of NO₃⁻ was 0.08 mg L⁻¹. At Nakabusa, the concentration of PO₄³⁻ was 0.28 mg L⁻¹, and that of NH₄⁺ ranged from 0.09 to 0.11 mg L⁻¹. NO₂⁻ was below detection limits in all examined hot spring effluents.

Constituents of Microbial Mats

Constituents of each microbial mat were examined spectrophotometrically and microscopically. We obtained no absorption peaks from the ST collected from 68 °C water (Fig. 2), which suggests that this ST did not contain any pigments in significant amounts. In contrast, we obtained clear absorption peaks at 680 nm and between 620 nm and 630 nm from CY (55 °C and 59 °C), which correspond to the absorption peaks of chlorophyll *a* and phycocyanin, respectively. However, CY taken from the 59 °C water showed a peak for bacteriochlorophyll *c* (740 ± 2 nm), which suggests that *Chloroflexus* or related green non-sulfur bacteria was present in that environment (Pierson and Castenholz 1991), and *Chloroflexus* sp. was observed under the microscope. We also obtained a peak at 740 ± 2 nm from olive-green and orange mats (BC), which corresponds to the absorption peak of bacteriochlorophyll *c*. Two absorption peaks at a long wavelength of more than 800 nm were obtained from a purple mat (PM) found at Nakanoyu hot spring, which suggests the presence of

photosynthetic bacteria other than cyanobacteria and *Chloroflexus*. Hanada et al. (2002) newly identified a filamentous photosynthetic bacterium from Nakabusa hot spring as *Roseiflexus castenholzii* gen. nov., sp. nov.; it had an optimum growth temperature of 50 °C and showed absorption peaks in vivo at 801 nm and 878 nm.

Examination of the DAPI-stained samples showed that sickle-shaped bacteria with cell length from 5 to 20 µm predominated in ST (Fig. 3). Unicellular and filamentous cyanobacteria about 5 µm long predominated in CY, which showed the red autofluorescence of chlorophyll *a*. Rod-shaped prokaryotic microorganisms about 2 µm long predominated in BC, and large rod-shaped prokaryotic microorganisms about 3 µm long predominated in PM.

Environmental Constraints of Microbial Mats

The different microbial mats observed in this study are listed in Table 2, together with their environmental parameters. In the hot springs so far examined in Japan, both ST and CY were present; generally ST was found in hotter locations than CY, but they both could be found where temperatures ranged from 51.1 °C to 63.2 °C. pH was somewhat high and dissolved oxygen concentration was significantly high in environments with CY compared with those with ST, reflecting the oxygen generated by the photosynthetic activity of the cyanobacteria. The highest dissolved oxygen concentration observed when ST was present was 1.81 mg L⁻¹. An increase in Eh represented oxygenation between the ST region and the CY region.

BC were found only at Yumata, where they coexisted with CY, but in areas with

slightly lower Eh. In addition to these mats, PM were found in a limited area at Nakanoyu.

Growth Experiment

The growth rate constant (μ) of the sickle-shaped bacteria was estimated by an in situ experiment. The estimates of cell number among the counting grids often had large standard deviations, from 17% to 175%, because the glass slides examined were not evenly covered by cells, and aggregates appeared often along with the pass of water on the glass slides. We counted more than 200 cells in total on 6H slides and more than 500 cells on 12H slides. Figure 4 shows the relation between the estimated μ and temperature or Eh of the in situ environment. Estimated μ tended to rise as temperature increased and Eh decreased, although in some cases no growth was found at temperatures less than 56 °C, irrespective of the value of Eh. The highest μ was estimated to 0.6 h⁻¹ (doubling time; 1.2 h) at 55.2 °C and Eh -68 mV. The result showed that sickle-shaped bacteria could grow between 50.7 and 57.7 °C and between -91 and 110 mV Eh, although temperatures higher than 58 °C were not tested in this experiment. Although in situ ST was restricted to locations with temperature higher than 51.1 °C and Eh lower than -25 mV, the sickle-shaped bacteria were often shown to grow in environments exceeding these limits. Cyanobacterial cells on glass slides immersed for 12 h accounted for 0% to 8% of all attached microorganism cells in this experiment.

Discussion

Yamamoto et al. (1998) demonstrated that the sickle-shaped bacterium, the major constituent of ST at all hot springs (Fig. 3), formed a major cluster with members of the *Aquifex-Hydrogenobacter* complex, which in turn are the most deeply branching bacteria on a phylogenetic tree based on 16S rRNA gene sequences. Maki (1991) suggested that ST develops in shallow sulfide-rich geothermal water streams with temperature ranging from 50 °C to 72 °C and pH between 6 and 9. In this study, we showed that the upper limit of temperature for ST was 77.2 °C, which is slightly higher than previously reported. Although Maki (1986) reported that the maximum DO concentration at which ST was able to develop was less than 1 mg L⁻¹, the maximum ambient DO concentration observed in the present study was 1.81 mg L⁻¹ (Table 2).

In hot spring effluents where ST and CY coexist, CY developed downstream from ST in all effluents with temperatures lower than 63.2 °C, whereas Ward et al. (1998) indicated that *Synechococcus lividus* formed microbial mats at an upper temperature limit of 74 °C in Yellowstone National Park, North America. The highest temperature at which cyanobacterial mat formation was found in this study of Japanese hot springs was 63.2 °C, which suggests that some other restrictive factor such as hydrogen sulfide may constrain the growth of more thermophilic cyanobacteria.

Microbial mats developed in hot spring streams displayed a stratified structure, in which the constituents of the deeper layer differed from those of the surface stratum when examined microscopically. However, we focused on the horizontal microbial distribution in this study rather than the vertical one to elucidate the environmental effect clearly, because we can easily find horizontal segregation of different types of mat in some Japanese hot springs. Thus, to examine the interactions between microbial mats and the environment in particular, we scrutinized the physico-chemical parameters at the boundaries of mats exposed to hot water effluent at their surfaces.

Figure 5 shows the distributions of the four microbial mat types (ST, CY, BC, and

PM) for each environmental parameter vs temperature. Overlapping boundary zones between ST and CY were found within the following ranges: temperature 51.1–63.2 °C; pH 7.53 and above; Eh –112 to –25 mV, and DO 0.25–1.81 mg L⁻¹. The effluents of both Nakanoyu and Nakabusa hot springs contained significant amounts of PO₄³⁻, NO₃⁻, and NH₄⁺. Thus, nitrogen and phosphorus do not seem to be limiting factors in the growth of microorganisms in the mats. It is clear that water temperature regulates the development of different mats (Fig. 5A–C). The development of the mats is also closely correlated with the Eh of the environment (Fig. 5A), which suggests that Eh controls the metabolic activity of microorganisms in the mats and that it must also be affected in turn by that activity. ST was present at temperatures higher than 51.1 °C, but only in a more reductive environment, that is, at Eh less than –75 mV; though ST was found together with CY even where the Eh was less than –25 mV. As the procedure in construction of overlapping zone is not cleared yet, a scenario is plausible that Eh was shifted by the development of CY onto ST which had previously grown there. CY, on the other hand, were found at temperatures below 63.2 °C, but only in more oxidized environments with Eh higher than –112 mV. Eh is directly affected by both DO and hydrogen sulfide concentrations. DO is increased by exposure to air and by photosynthetic activity of cyanobacteria. The hydrogen sulfide concentration decreases as a result of volatilization in flowing effluent and biological oxidation. Hydrogen sulfide must restrict the development of cyanobacterial mats (Castenholtz 1976), whereas ST requires a significant amount of hydrogen sulfide for its growth (Maki 1991). Thus, the hydrogen sulfide concentration can be a major factor controlling the development of both ST and CY. However, as hydrogen sulfide-tolerant and more thermophilic cyanobacteria were not found in the examined Japanese hot springs, temperature, Eh, and DO were shown here to be good indicators and regulators of the boundary environment in flowing systems.

In situ pH depended on the source of the effluent. Production of H₂SO₄ by the

oxidation of sulfur theoretically decreases the pH of the environment, and lowering the temperature increases carbon dioxide dissolution, which also decreases pH, but in situ pH actually tended to increase with the flow of effluent, probably reflecting the oxygen generated by the photosynthetic activity of cyanobacteria (Fig. 5B).

In situ examination revealed that the boundary between ST and CY lay between -112 and -25 mV Eh (Fig. 5A). The results of the growth experiments, however, showed that sickle-shaped bacteria grew from Eh -96 to 110 mV (Fig. 4). The method used to estimate the growth of sickle-shaped bacteria was primitive and was based on an increase the number of attached cells on a glass slide within a given period. The cell count was confirmed only by cell shape and staining with DAPI, which stains DNA irrespective of species. Thus, whether the increase in cell number on the glass slide could legitimately be ascribed to growth is as yet uncertain. However, no other method is yet available to estimate the in situ growth rate of mat forming thermal chemolithotrophic bacteria that are not yet able to be cultivated. This study may lead the field of extreme microbial ecology from mere findings of existence and the elucidation of phylogeny on the basis of DNA to an understanding of the activity and function of extremophiles in a given ecosystem. The results suggest that sickle-shaped bacteria have the potential to grow in a somewhat more oxidized environment than that observed in situ, although controversy remains concerning the source of hydrogen sulfide and its impact on Eh. After incubation for 12 h, cyanobacterial cells attached to glass slides accounted for less than 8% of the total number of attached cells. Thus, the effect on the environment of cyanobacterial activity on glass slides proved to be weaker than that found in situ. The experiment suggests that if there is any direct effect of cyanobacteria, sickle-shaped bacteria could develop ST and thus alter the in situ environment. The actual lower boundary of ST was found to be restricted by interaction or competition with cyanobacteria.

Chloroflexus can coexist with both ST and CY (Fig. 2), although BC is also found in

sites similar to the boundary zone between ST and CY (Fig. 5). The flexible metabolic behavior of *Chloroflexus* (Pierson and Castenholz 1991) may allow this. On the other hand, ST and CY rarely coexist, although they may be found adjacent to each other. Sickie-shaped bacteria do not construct mats in cyanobacterial habitats, even though they have growth potential within the boundaries of CY. This suggests that an exclusive interaction sustains the boundary between these two ecosystems.

Aquificales, which includes the thermophilic sulfur oxidizer of this study, is widely distributed in geothermal and hydrothermal environments (Reysenbach et al. 2000). Bacteria genetically similar to our sulfur-turf-constructing thermal sulfur oxidizer have been described recently from Iceland (Skirnisdottir et al. 2000), and a candidate similar to ours was previously nominated from Yellowstone National Park (Hugenholtz et al. 1998). We carried out observations and sampling in Yellowstone in 1999 and 2000 and found a genetically similar thermophilic sulfur oxidizer in some sites in Yellowstone. However, it did not form turf but grew as streamers (Yamamoto Hiraishi and Kato, in preparation). The environmental conditions where genetically similar thermophilic sulfur oxidizers were found are summarized in Table 3. All parameters of the hot springs at Yellowstone – temperature, pH, Eh, DO, and H₂S – overlapped with those of the Japanese hot springs. Measurements in Iceland were also very similar. Thus, temperature and other parameters relevant to the sulfur-oxidizing energy-generating system define clearly the habitat of thermophilic sulfur oxidizers, whether they construct dense mats or appear as hair-like streamers. In addition, if cyanobacterial mats are present, they may restrict the habitat of sulfur-oxidizing bacteria through competition. Under the restricted environmental conditions of chemolithotrophic thermophiles, genetically similar bacteria appear under a certain subset of those conditions. The environment discussed here is, furthermore, presumed to resemble that of the early Archean stage of the evolution of life on Earth (Reysenbach and Cady 2001).

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TABLE 1 Environmental parameters of the effluent at the source of each hot spring

Hot spring	Date	n	Temperature (°C)	EC (mS m ⁻¹)	pH	Eh (mV)	DO (mg L ⁻¹)	S ²⁻ (mg L ⁻¹) ^c
Nakafusa	1998.10.9	17	53.6-62.5	36.4-45.5	8.17-8.44	-136 - -52	0.42-1.66	2-3
	1998.10.16	17	51.8-64.9	36.8-48.9	8.00-8.18	-116 - -40	0.39-1.47	2.5-3
	1998.10.23	13	55.8-64.5	31.8-34.2	8.17-8.26	-82 - +11	0.47-1.43	ND ^b
	1998.10.30	13	52.8-59.2	40.0-44.8	8.28-8.36	-111 - -17	0.35-1.45	2
Nakanoyu	1998.5.9	1	83.6-85.1	102.8-106.4	6.52	-205 - -195	0.22-0.27	6
	1998.11.12	1	89.2	105	6.22	-180	BD ^a	10
Yumata	1998.8.27	1	79.7	183.7	6.31	-170	BD ^a	25

^a Below detection limit.^b No data.^c Determined with a simple tube kit

TABLE 2 Environmental constraints on the different microbial mats at Japanese hot springs

Mat type	Range of environmental parameters for 4 mat types					Appearance of 4 mat types in 3 examined hot springs ^a		
	Temperature (°C)	pH	EC (mS m ⁻¹)	Eh (mV)	DO (mg L ⁻¹)	Nakafusa	Nakanoyu	Yumata
Sulfur-turf (ST)	51.1 - 77.2	6.45 - 8.41	36.4 - 181.4	-205 - -25	< 1.81	+	++	++
Cyanobacterial mat (CY)	40.0 - 63.2	7.53 - 8.48	27.0 - 133.7	-112 - +173	0.25 - 5.25	++	++	+
Bacteriochlorophyll c - containing mats (BC)	53.5 - 60.3	7.73 - 7.94	164.7 - 174.3	-143 - -89	0.49 - 2.02	-	-	++
Purple colored mat (PM)	48.8	7.71	104.1	-67	1.85	-	+	-

^a ++, the mat existed in a wide region. +, the mat existed in a limited region. —, the mat did not exist.

TABLE 3 Comparison of three environments where colorless sulfur bacteria have been found

Hot spring	Temperature (°C)	pH	Eh (mV)	DO (mg L ⁻¹)	H ₂ S (mg L ⁻¹)
Japanese hot springs	51.1 - 77.2	6.45 - 8.41	-205 - -25	< 1.81	0.07 - 9.37 ^b
Yellowstone	58.1 - 72.3	6.19 - 7.62	-133 - -59	0.35 - 1.59	< 10.6 ^b
Iceland (Skirnisdottir et al. 2000)	67	6.7	ND ^a	ND ^a	12 ^c

^a No data.^b Determined by spectrophotometry^c Determined by mercury acetate titration



Figure 1. Co-existence of sulfur-turf (ST; white) and cyanobacterial mats (CY; dark green) with distinct boundaries between them on the wall of a debris dam at Nakafusa hot springs. ST growth is initiated at several points where sulfide rich effluent flows from horizontal cracks. The white ST is about 10 cm wide.

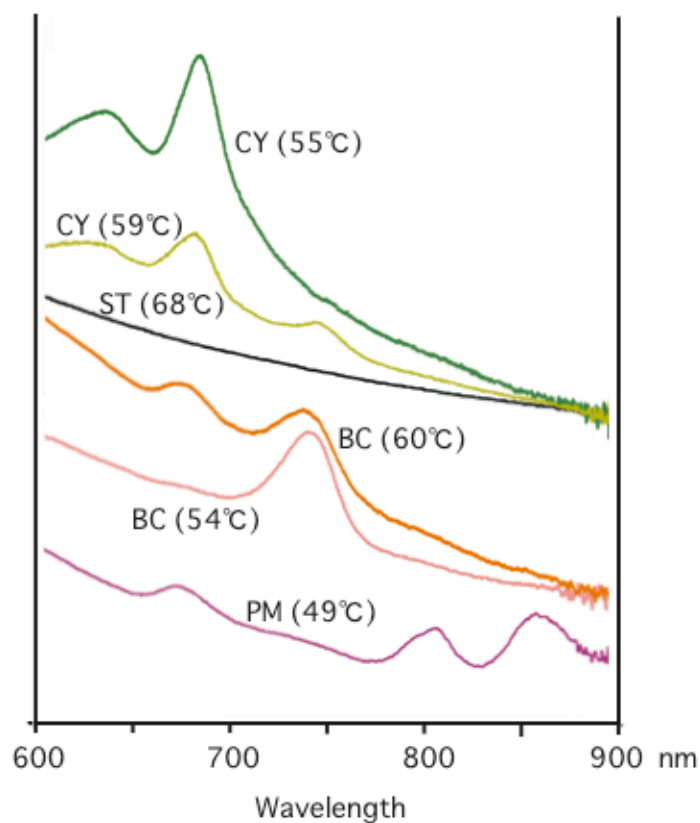


Figure 2. Absorption spectra of hot spring microbial mats. CY (55 °C), cyanobacterial mat growing at 55 °C at Nakafusa hot spring. CY (59 °C), cyanobacterial mat growing at 59 °C at Nakafusa hot spring; ST (68 °C), sulfur-turf growing at 68 °C at Yumata hot spring; BC (60 °C), bacteriochlorophyll *c*-containing mat growing at 60 °C at Yumata hot spring; BC (54 °C), bacteriochlorophyll *c*-containing mat growing at 54 °C at Yumata hot spring; PM (49 °C), purple mat growing at 49 °C at Nakanoyu hot spring.



— 10 μ m

Figure 3. Epifluorescence microscopic image of large sickle-shaped bacteria stained with 4',6-diamidino-2-phenylindole (DAPI). Length of cells is about 20 μ m.

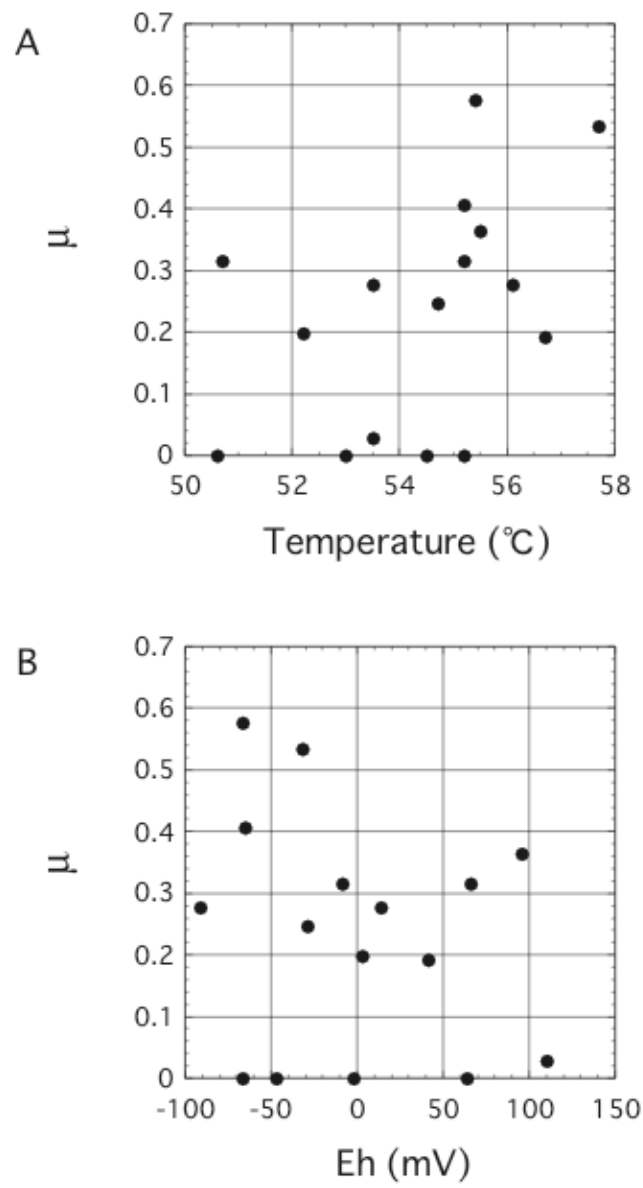
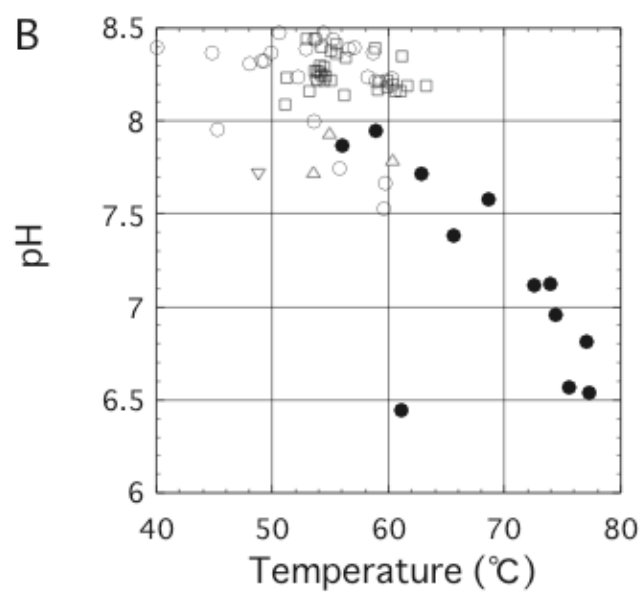
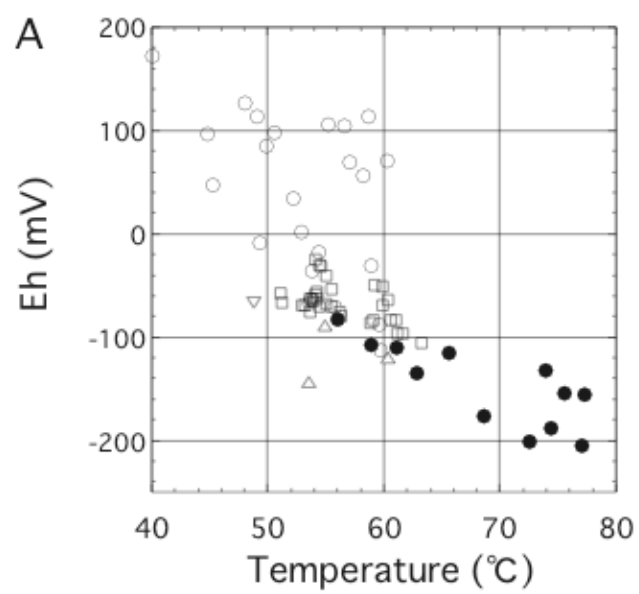


Figure 4. Estimated growth rate constant (μ) of sickle-shaped bacteria compared with temperature (A) and Eh (B).



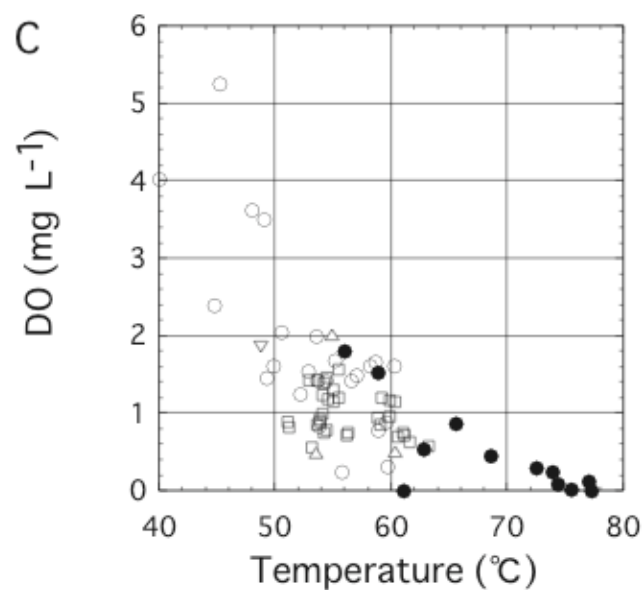


Figure 5. Distribution of microbial mats in the Japanese hot spring effluents examined. Distribution is shown in relation to (A) temperature–Eh, (B) temperature–pH, and (C) temperature – dissolved oxygen (DO). ●, sulfur-turf (ST, $n = 12$); ○; cyanobacterial mat (CY, $n = 24$); △, bacteriochlorophyll *c*-containing microbial mats (BC, $n = 3$). ▽; purple microbial mat (PM, $n = 1$); □, zone of coexistence of ST and CY at Nakafusa hot spring ($n = 32$).