FACTORS IN HABITAT PREFERENCE IN SITU OF SULFUR-TURFS GROWING IN HOT SPRINGS EFFLUENTS: DISSOLVED OXYGEN AND CURRENT VELOCITIES¹

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Field surveys of hot springs were carried out at four Spas. Observations were made on the temperature, pH, dissolved oxygen and sulfide concentrations, current velocities, and the habitats of three (A, B, C) types of sulfur-turfs. The B-type (rods) was not present, while the A-type (sausage-shaped bacteria) grew only in the streams. In contrast, the C-type (filamentous bacteria) inhabited only the pools of hot spring water. Ranges of dissolved oxygen concentrations in situ were 6 to 31 μM for the A-type and 6 to 110 μM for the C-type sulfur-turfs. The sausage-shaped bacteria (main bacterial component of the A-type sulfur-turf) had peritrichous flagella which were not present in the filamentous C-type bacteria. It was suggested that the A-type sulfur-turf preferred the stream to the pool because of this bacteria's low level and narrow range of oxygen requirement and its ability to form aggregates by intertwining flagella, even in flowing water. In contrast, the C-type preferred the pool rather than the stream due to its wide range of oxygen requirement and its inability to form colonies in rapidly flowing water.

Sulfur-turf consists of aggregates of sulfur-oxidizing bacteria and elemental sulfur particles adhering to them. It grows in thermal waters (from 45 to 72°C) containing some dissolved sulfides (under 40 ppm or 1.2 mM) and having a neutral pH (between 5 and 9) (1).

In 1897, MIYOSHI named it "Schwefelrasen" (sulfur-turf) after its appearance and studied its physiological and ecological characteristics (2). EMOTO (1941) investigated the distribution of the sulfur-turf in Japanese hot springs (3). Based on the main bacterial components, he proposed three types of the sulfur-turf: A-type

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Fig. 1. Schematic representations of the side views of the stream and the pool. They are the basic constitutions of the effluent of a hot spring. The stream-bed, the stream-edge, and the surface of the pool are the habitats of the sulfur-turfs (**).

(mainly consisting of sausage-shaped bacteria), B-type (rods), and C-type (filamentous bacteria) (4).

One representative group of colorless sulfur bacteria is the so-called "gradient bacteria" (5). One of these bacteria's prominent features is that they form unique communities across the sulfide-oxygen interface. BAAS-BECKING labelled the community "sulphuretum" (6). Some gradient bacteria form aggregates or colonies, and according to their appearance they are called the veil (*Thiovulum*) (7, 8), the mat (*Beggiatoa*) (9), or the streamer (*Thermothrix thiopara*) (10). The sulfur-turf is one such bacterial aggregate.

While these gradient bacteria have been tentatively classified, their ecology remains poorly understood (5, 11). From an ecological point of view, the pool and the stream as well as the stream-bed and the stream-edge are the basic components of the effluent of hot springs. Each of these constitutes a unique habitat for microorganisms (Fig. 1). CALDWELL et al. (1984) reported the mechanism of cell positioning of *Thermothrix thiopara* on hot spring tufa in relation to the molar ratio of sulfide to oxygen in the reducing geothermal ground-water mixed with the oxidizing atmosphere (10). I report here the significance of both the dissolved oxygen concentrations and the current velocities on the growth of sulfur-turfs and their habitat preference in situ.

MATERIALS AND METHODS

Field surveys. The field surveys were carried out from 1979 to 1982 at four Spas in the Tohoku district: Magoroku, Ganiba, Tsuru-no-yu (in Akita Ken), and Geto (Iwate Ken). Names of the hot springs in the four Spas are after NAKADATE et al. (12).

Measurements of temperature, pH, and dissolved sulfide concentrations. Temperature was measured with an alcohol thermometer. The pH measurements were carried out in situ using a glass electrode pH meter for the field survey (TOA model HM-12A). Dissolved sulfide concentrations were estimated using a commercially available reaction tube for sulfide ion (Kitazawa Sangyo Co., No. 211L).

This glass tube is packed with a mixture of silica gel and lead acetate. When the tube is dipped into the water containing sulfide ions, a definite volume of water penetrates into the mixture, and the sulfide ions react with the lead acetate producing the brown color of lead sulfide.

Dissolved oxygen concentrations. A 60 ml disposable syringe tipped with a flexible plastic tube about 10 cm long was used as the sampling tool. The samples of hot water were collected in BOD bottles of 100 ml volume. Three 60 ml aliquots were transferred to each bottle resulting in an overflow of 80 ml of water. Immediately, these were sealed with glass stoppers and tightly covered with vinyl film and then brought back to the laboratory. Dissolved oxygen concentrations were determined using an oxygen meter (YSI model 57).

Habitat of the sulfur-turfs and current velocities. Observations were first made to determine whether the sulfur-turf grew in the pool or the stream. Then,

Fig. 2. Schematic representations of the side views of the hot springs and the occurrence of the A- (●●●) and C-type (○○○) sulfur-turfs.

This figure shows the arrangements of the position of the source, the pool, the stream, relative to the occurrence of the sulfur-turfs. The Teizo-no-yu and the Mukai-no-yu had the same arrangement. A; Ganiba Spa, B; Teizo-no-yu and Mukai-no-yu Springs in the Geto Spa, C; No. 2 Spring in the Magoroku Spa, D; Karematsuzawa Spring in the Geto Spa, E; No. 2 Spring in the Tsuru-no-yu Spa.

Fig. 3. Growth of the A- and C-type sulfur-turfs at the Mukai-no-yu Spring in the Geto Spa.

A: The A-type sulfur-turf (a) grew only in the stream overflowing from the pool. In contrast, the growth of the C-type (b) occurred only in the pool. B: The C-type sulfur-turf was attached to the walls of the pool within a few centimeters from the water surface and extended its filaments (arrows).

depending on these results, further observations were made to determine whether the sulfur-turf grew on the surface or the bottom of the pool, or on the stream-bed or at the stream-edge (Fig. 1). I also recorded the current velocities for each of the pools and the streams.

Staining. The Toda method was applied for staining the flagella of the sausage-shaped bacteria.

RESULTS

Habitat of the sulfur-turfs, current velocities, temperature, and pH of the hot spring waters

Figure 2 contains schematic representations of the side views of the hot springs and the occurrence of the A- and C-type sulfur-turfs. At the Ganiba Spa (Fig. 2A), the water of the source formed a pool (1 × 1 m) and then flowed down a concrete gutter and was distributed to baths by wooden gutters. The A-type sulfur-turf grew conspicuously on the bed and the side walls of the concrete and wooden gutters, but there was no growth in the pool or baths.

At the Teizo-no-yu and the Mukai-no-yu Springs in the Geto Spa (Fig. 2B), the

Table 1. Habitat of the sulfur-turfs and the current velocity, temperature, and pH of the hot spring waters.

Spa	Spring	Distance from the source (m)	Type of sulfur-turfs	Habitat	Current velocity (cm/sec)	Temperature (°C)	pH
Magoroku	No. 1	0	A	stream	20–30	60	6.7
	No. 2	0 3	A C	stream pool	20–30 1–5	63 55–58	6.7 6.9–7.0
Ganiba	Ganiba	0	no growth	pool	— ^a	53	7.9
		2	A	stream	100	52	8.0
		3	A	stream	50	52	8.1
Tsuru-no-yu	No.2	0	no growth	stream	—	60	6.4
		0.3	A	stream	45	—	—
		0.8	A	stream	45	59	6.5
Geto	Karematsumazawa	0	no growth	stream	—	72	6.3
		0.4	A	stream	80	—	—
		1.1	A	stream	10	71	—
		2.5	A, C	stream	10	68	6.4
	Mukai-no-yu	0	C	pool	1–5	54	6.0–6.4
		0.2	A	stream	50	54	6.2
	Teizo-no-yu	0	C	pool	1–3	61	6.0
		1	A	stream	30	58	—

^a “—” indicates no determination.

Table 2. Type of sulfur-turfs, dissolved sulfide and oxygen concentrations, and molar ratio of sulfide to oxygen.

Spa	Spring	Distance from the source (m)	Type of sulfur-turfs	Dissolved sulfide (μM)	Dissolved oxygen (μM)	Molar ratio HS^-/O_2
Magoroku	No. 1	0	A	90	12	7.5
		0	A	90	16 ^a	5.6
		3	C	60	110 $\downarrow$	0.5
Ganiba	Ganiba	0	no growth	140	12–16 $\downarrow$	8.8–12
		2	A	90	12	7.5
		3	A	60	19–31 $\downarrow$	1.9–3.2
Tsuru-no-yu	No. 2	0	no growth	720	—	—
		0.3	A	— ^b	—	—
		0.8	A	600	—	—
Geto	Karematuzawa	0	no growth	150	—	—
		0.4	A	150	6–12 $\downarrow$	13–25
		1.1	A	90	16	5.6
		2.5	A, C	60	25–31 $\downarrow$	1.9–2.4
	Mukai-no-yu	0	C	90	—	—
		0.2	A	—	—	—
	Teizo-no-yu	0	C	90	6	15
		1	A	—	6	—

^a Arrow indicates the gradient of dissolved oxygen along the effluents.

^b “—” indicates no determination.

emerging hot water formed a small pool and then overflowed at one end of the pool. The A-type sulfur-turf proliferated only in the stream of this overflow and the C-type grew only in the pool. Figure 3 shows the growth of the A- and C-type sulfur-turfs at the Mukai-no-yu Spring.

At the Magoroku Spa, there were several hot springs, and two (No. 1 and No.2) were inhabited by the A-type sulfur-turf (Fig. 2C). The two effluents from these springs flowed down on rocks for 2 to 3 m and then flowed into one large pool (about 1×10 m). The C-type sulfur-turf proliferated in this pool attaching to floating objects and stretching its filaments to the water surface; however, the A-type did not grow there.

Table 1 shows the habitat of the sulfur-turfs, current velocity, temperature, and pH of the hot spring waters. The velocities of the streams inhabited by the A-type sulfur-turf ranged from 20 (Magoroku Springs) to 100 cm/sec (Ganiba Spring). Velocities in the pools in which the C-type grew were estimated to be 1 to 5 cm/sec (Teizo-no-yu, and Magoroku No. 2 Springs). The range of temperature at which the A-type sulfur-turf grew (52 to 72°C) was similar to that of the C-type (54 to 68°C). In contrast, the pH range of the A-type sulfur-turf was wider (6.2 to 8.1) than that of the C-type (6.0 to 7.0). The highest pH values of 7.9 to 8.1 were recorded at

Ganiba Spring where the A-type sulfur-turf grew.

Dissolved oxygen and sulfide concentrations, and the type of sulfur-turfs

As shown in Table 2, the range of dissolved oxygen concentrations in the water where the A-type sulfur-turf grew was from $6\ \mu\text{M}$ (Karematuzawa and Teizo-no-yu Springs) to $31\ \mu\text{M}$ (Karematuzawa and Ganiba Springs), and that of the C-type was from $6\ \mu\text{M}$ (Teizo-no-yu Spring) to $110\ \mu\text{M}$ (Magoroku No. 2 Spring). On the other hand, the ranges of dissolved sulfide concentrations for the A-type were from $60\ \mu\text{M}$ (Ganiba) to $600\ \mu\text{M}$ (Tsuru-no-yu No. 2), and for the C-type $60\ \mu\text{M}$ (Magoroku No. 2) to $90\ \mu\text{M}$ (Mukai-no-yu and Teizo-no-yu). The highest value of dissolved sulfide was recorded at Tsuru-no-yu No. 2 Spring where the A-type grew. Changes in the sulfide concentrations during the 4-year sampling period were within 0.5 ppm or $15\ \mu\text{M}$ at all the springs.

Growth of the A-type sulfur-turf in the proximity of the sources

At the Karematuzawa Spring in the Geto Spa (Figs. 2D and 4A), the hot water flowed down about 30 cm in a stream and then spread out on a flat concrete bed where a large amount of calcium carbonate called "Yubana" (calcareous sinter) precipitated. On the stream bed (Fig. 4A), the A-type sulfur-turf did not occur from

Fig. 4. Growth of the A-type sulfur-turfs in the proximity of the sources.

A₁ and A₂: Karematuzawa Spring in the Geto Spa, B₁ and B₂: No. 2 Spring in the Tsuru-no-yu Spa. a: source, b: growth of the A-type sulfur-turf on the edge of the stream, c: growth on the stream-bed.

the source (a) to 30 cm downstream, but it grew conspicuously (c) on the concrete bed more than 30 cm downstream from the source. In contrast, on the stream-edge, the growth (b) occurred at about 20 cm downstream from the source. The C-type sulfur-turf grew with the A-type at the lower end of the concrete bed (Fig. 2D).

At the No. 2 Spring in the Tsuru-no-yu Spa (Figs. 2E and 4B), the A-type sulfur-turf grew visibly on the edge of the stream (b) down to about 20 cm from the source, but on the stream-bed (c) growth did not begin until about 70 cm downstream from the source (a).

DISCUSSION

Relationship between the growth of the sulfur-turfs and dissolved oxygen concentrations

As shown in Table 2, a gradient of dissolved oxygen was detected along the effluents (Magoroku No. 2, Ganiba, and Karematsuzawa Springs). The dissolved oxygen concentrations were lowest at the sources and gradually increased after mixing with the air. Therefore, the response to this dissolved oxygen by the sulfur-turfs can be judged from the positions of their growth on the effluent.

The A-type sulfur-turf was not found on the stream-beds in the proximity of the sources at the Karematsuzawa Spring and the No. 2 Spring in the Tsuru-no-yu Spa (Fig. 4). Since the members of the A-type sulfur-turf are sulfur-oxidizing bacteria (1), they require molecular oxygen to oxidize sulfides or elemental sulfur. As the water at the sources was just coming from the depth of the earth, it should have had little dissolved oxygen. Therefore, the absence of growth of the A-type sulfur-turf on the stream-beds in the proximity of the source was probably due to an insufficiency of dissolved oxygen. Indeed, the A-type sulfur-turf grew visibly on the edge of the stream about 20 cm down from the source, where oxygen could be readily supplied from the air.

In contrast, the C-type sulfur-turf grew in pools at the source (Mukai-no-yu and Teizo-no-yu Springs) (Fig. 2B), and it also grew in a pool far from the source (Magoroku No. 2 Spring) (Fig. 2C). Thus, the range of dissolved oxygen concentrations where the C-type grew (6 to 110 μM) was considerably wider than that for the A-type (6 to 31 μM) (Table 2).

CALDWELL et al. reported that the streamers of *Thermothrix thiopara* positioned themselves at the sulfide-oxygen interface, where the range of the molar ratio of sulfide to oxygen was between 0.3 and 0.8 (10). They showed that these values corresponded to the optimum stoichiometric proportion (0.5) necessary for sulfur metabolism (10). The range of dissolved oxygen concentration for the sulfur-turfs (from 6 to 110 μM) (Table 2) was comparable to that for *Thermothrix* (20 to 69 μM) (10). On the other hand, the level of dissolved sulfide for the sulfur-turfs (from 60 to 600 μM) was considerably higher than that for *Thermothrix* (8.2 to 24.3 μM) (10). In line with those values, the calculated range of the molar ratio of sulfide to oxygen was also higher in the sulfur-turfs (0.5 to 25) (Table 2). As these

Fig. 5. The intertwining flagella (arrows) of the sausage-shaped bacteria. Flagella were stained by the Toda method.

values were considerably higher than that of the optimum stoichiometric proportion, (0.5), it appears that the sulfur-turfs grew in conditions where there was an overabundance of sulfide, and that the dissolved oxygen concentration was the limiting factor.

Apparently, the concentration of dissolved oxygen is the primary factor in determining the habitat of the A- and C-type sulfur-turfs in sulfide-rich effluents, if high concentrations of sulfide are not toxic for them. Judging from the level of dissolved oxygen (under $31 \mu\text{M}$ or 1 ppm), the main bacterial component of the A-type sulfur-turf (sausage-shaped bacteria) may be a microaerophile.

Flagella of sausage-shaped bacteria

The sausage-shaped bacteria, which are the main bacterial component of the A-type sulfur-turf, had peritrichous flagella (2, 4, 13) (Fig. 5). These flagella should be advantageous for the bacteria in forming aggregates especially in rapidly flowing water, and the importance of bacterial flagella in adhesion and aggregation has been indicated by several authors (14–16). In particular, an inability of the C-type sulfur-turf to form aggregates in flowing water may restrict its habitat to the pool.

Factors in habitat preference of the sulfur-turfs

The A-type sulfur-turf grew only in streams. In contrast, the C-type inhabited only pools. Furthermore, the two types of sulfur-turfs grew in one effluent at Magoroku, Mukai-no-yu, and Teizo-no-yu Springs (Table 1). In these effluents, the pool and the stream were only a few meters apart (Fig. 2). Due to this close

proximity, the temperature and pH of the water in the pool and the stream were very similar (Table 1), and for the same reason, the major ion concentrations (excluding gaseous components) were probably also similar. As for the gaseous components in these hot springs, hydrogen sulfide and carbon dioxide were released into the air and their concentrations gradually decreased moving downstream away from the source. However, the difference in dissolved sulfide concentrations in the pools and streams was very small, as well as the carbon dioxide concentrations as indicated by the pH values which were almost the same for the pools and streams (Table 2). Therefore, given the similarities in temperature, pH, major ion concentrations, and gaseous components in the pool and stream water, it is doubtful that these factors played a role in the habitat preference of sulfur-turfs. At the Ganiba Spring, there was no growth of the C-type sulfur-turf at the pool, distributor, and the baths. This may be due to the high pH value of the hot spring water.

The range of the velocities of the streams inhabited by A-type sulfur-turf was from 20 (Magoroku Springs) to 100 cm/sec (Ganiba), and that of the pools in which the C-type grew was estimated to be only 1 to 5 cm/sec. Accordingly, the difference in the pool and stream habitats was interpreted as a difference in the current velocity. At the Karematsuzawa Spring, the A- and C-type sulfur-turfs grew together in the stream on the concrete bed (Fig. 2D). This may be due to the slow movement of the water (10 cm/sec) (Table 1).

In a rapid stream, low oxygen tensions are probably maintained by the continuous supply of fresh hot water from the source. In a pool, on the other hand, if the turnover rates of the water were low, the water would be gradually oxygenated by the diffusion of the air from the surface. This is indicated by the $110\text{ }\mu\text{M}$ of dissolved oxygen in the pool at the Magoroku No. 2 Spring (Table 2).

The data suggest the following conclusions: The A-type sulfur-turf inhabits the stream and not the pool because of its low and narrow range of oxygen requirement and its ability to form aggregates in flowing water. In contrast, the C-type sulfur-turf inhabits the pool rather than the stream due to its wide range of oxygen requirement and its inability to form colonies in rapidly flowing water.

The flow rate of the spring and the depth or width of the stream may influence the distribution indirectly by modifying dissolved oxygen, pH, and temperature. In contrast, the current velocity, in addition to the above indirect effect, may directly determine the distribution of sulfur-turfs by affecting the possibility of successful attachment of cells to substrate. In order to study the mechanisms of the current velocity effect on the distribution of sulfur-turfs, experimental approaches are necessary.

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REFERENCES

- 1) Y. MAKI, K. NAKADATE, and R. FUJITA, *Iwate Ika Daigaku Kyoyobu Kenkyu Nenpo*, **18**, 41 (1983).
- 2) M. MIYOSHI, *J. Coll. Sci. Imp. Univ. Tokyo*, **10**, 143 (1897).
- 3) Y. EMOTO, *Nihon Gakujutsukyokai Hokoku*, **16**, 425 (1941).
- 4) Y. EMOTO, *Seitaigaku Kenkyu*, **8**, 94 (1942).
- 5) B. B. JØRGENSEN, *Philos. Trans. R. Soc. London, Ser. B*, **298**, 543 (1982).
- 6) L. G. M. BAAS-BECKING, *Ann. Bot.*, **39**, 613 (1925).
- 7) C. O. WIRSEN and H. W. JANNASCH, *J. Bacteriol.*, **136**, 765 (1978).
- 8) B. B. JØRGENSEN and N. P. REVSBECH, *Appl. Environ. Microbiol.*, **45**, 1261 (1983).
- 9) B. B. JØRGENSEN, *Mar. Biol.*, **41**, 19 (1977).
- 10) D. E. CALDWELL, T. L. KIEFT, and D. K. BRANNAN, *Geomicrobiol. J.*, **3**, 181 (1984).
- 11) J. G. KUENEN, *Plant Soil*, **43**, 49 (1975).
- 12) K. NAKADATE, R. FUJITA, and K. MATSUDA, *Iwate Ika Daigaku Kyoyobu Kenkyu Nenpo*, **16**, 1 (1981).
- 13) Y. MAKI, *Iwate Ika Daigaku Kyoyobu Kenkyu Nenpo*, **16**, 47 (1981).
- 14) W. A. SCHEFFERS, the late W. E. DE BOER, and A. M. LOOYAARD, *In Aquatic Microbiology*, ed. by F. O. SKINNER and J. M. SHEWAN, Academic Press, London (1977), p. 351.
- 15) R. D. SJOBLAD and R. N. DOETSCH, *Curr. Microbiol.*, **7**, 191 (1982).
- 16) S. KJELLEBERG, *In Microbial Adhesion and Aggregation*, ed. by K. C. MARSHALL, Springer-Verlag, Berlin (1984), p. 51.