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Dense Community of Hyperthermophilic Sulfur-Dependent Heterotrophs in a Geothermally Heated Shallow Submarine Biotope near Kodakara-Jima Island, Kagoshima, Japan

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Microbial communities in marine hydrothermal sediments (0 to 30 cm deep) in an inlet of Kodakara-Jima Island, Kagoshima, Japan, were studied with reference to environmental factors, especially the presence of amino acids. The study area was shallow, and the sea floor was covered with sand through which hot volcanic gas bubbled and geothermally heated water seeped out. The total bacterial density increased with depth in the sediments in parallel with a rise in the ambient temperature (80°C at the surface and 104°C at a depth of 30 cm in the sediments). As estimated by most-probable-number studies, hyperthermophilic sulfur-dependent heterotrophs growing at 90°C dominated the microbial community (3×10^7 cells · g of sediment^{-1} at a depth of 30 cm in the sediments), followed in abundance by hyperthermophilic sulfur-dependent facultative autotrophs (3.3×10^2 cells · g of sediment^{-1}). The cooler sandy or rocky floor surrounding the hot spots was covered with white bacterial mats which consisted of large *Beggiatoa*-like filaments. Both the total organic carbon content, most of which was particulate (75% in the surface sediments), and the amino acid concentration in void seawater in the sediments decreased with depth. Amino acids, both hydrolyzable and free, constituted approximately 23% of the dissolved organic carbon in the surface sediments. These results indicate that a lower amino acid concentration is probably due to consumption by dense populations of hyperthermophilic sulfur-dependent heterotrophs, which require amino acids for their growth and thus create a gradient of amino acid concentration in the sediments. The role of primary producers, which supply essential amino acids to sustain this microbial community, is also discussed.

In the marine environment, habitats for hyperthermophiles are generally limited to active hydrothermal zones, which are widespread in the oceans (5). Hyperthermophiles from marine sources include methanogens, sulfate reducers, elemental sulfur (S^0)-dependent facultative autotrophs, and S^0-dependent heterotrophs (38). Most belong to the domain *Archaea* (40). A large number of hyperthermophilic S^0-dependent heterotrophic archaea, which are the major hyperthermophiles known at present, are limited to growth on complex proteinaceous substrates such as yeast extract (7, 14, 22, 23, 33, 41–44). We have previously shown that a growth dependency on amino acids characterizes not only deep-sea isolates, such as *Pyrococcus* strain GB-D and *Desulfurococcus* strain SY (12), but also shallow-sea isolates, such as *Pyrococcus furiosus*, *Thermococcus celer*, and three others from the site of the present study (11). These results indicate that amino acids play an important role in supporting hyperthermophilic S^0-dependent heterotrophs in marine hydrothermal habitats.

Light-independent primary production in the ecosystem surrounding deep-sea hydrothermal vents has been reported (17, 18). Massive numbers of mesophilic sulfur-oxidizing bacteria are the primary producers, gaining energy from the oxidation of geothermally reduced sulfur compounds and thus supporting the dense community of invertebrates that subsist on them on the sea floor near the vents (20, 25). On the other hand, the presence of strictly anaerobic, autotrophic hyperthermophiles such as *Methanococcus jannaschii* indicates that these organisms may be the primary producers in high-temperature habitats, because the geothermally heated environments are strictly anaerobic (38). However, little information on the microbial community of the hyperthermophiles and their distribution in high-temperature habitats is available, and thus, nutritional interactions among hyperthermophiles are unknown.

In the present study, we aimed to describe the distributions and population densities of both autotrophic and heterotrophic hyperthermophiles and to clarify their relationships with environmental factors, including concentrations of organic carbon and of amino acids.

MATERIALS AND METHODS

Sampling. Samples were collected at a marine hydrothermal field (depth of 3 to 10 m) in an inlet of Kodakara-Jima Island (29°13′N, 129°20′E) during a cruise of the research vessel *Sohgen-maru* in 1993 (Fig. 1). Evolving volcanic gas bubbles were collected into 2-liter gas collection bags (TEDLAR BAGS; Iuchi, Osaka, Japan) equipped with a funnel. Seawater samples (approximately 3 to 5 liters) and hot sediment samples (approximately 50 g for microbiological study and approximately 1 kg for chemical analysis) were collected from three sites in the inlet by scuba diving, using sterile screw-cap sealed plastic bottles or plastic bags. The seawater samples were collected at 100 and 10 cm above the gas-evolving spots, and the sediment samples were collected at the surface (0 to 5 cm), and at depths of 10 and 30 cm. After collection, the samples were cooled on ice in a cooler box and brought back to the laboratory on the *Sohgen-maru*. For chemical analyses, both nonfiltered and filtered samples were frozen at −80°C until analyzed.

Environmental conditions. The flow rate of the evolving volcanic gas from a single hot spot was measured in triplicate by trapping gas into a seawater-filled volumetric cylinder equipped with a funnel (8.8 cm in diameter) for 1 to 3 min. Temperatures were measured by using a mercury-filled Celsius thermometer (0 to 200°C). Other environmental factors were analyzed in the ship's laboratory. The pHs of the samples were measured with a pH meter (Toa Electronics Ltd., Tokyo, Japan), and salinities were measured with a salinometer (ATAGO S/Mill; Atago Co. Ltd., Tokyo, Japan) after calibration against 30‰ KCl. Dissolved sulfide was analyzed by using lead acetate paper with an H_2S analysis kit (Kyoritsu Chemical-Check Laboratory Corp., Tokyo, Japan). For determination of

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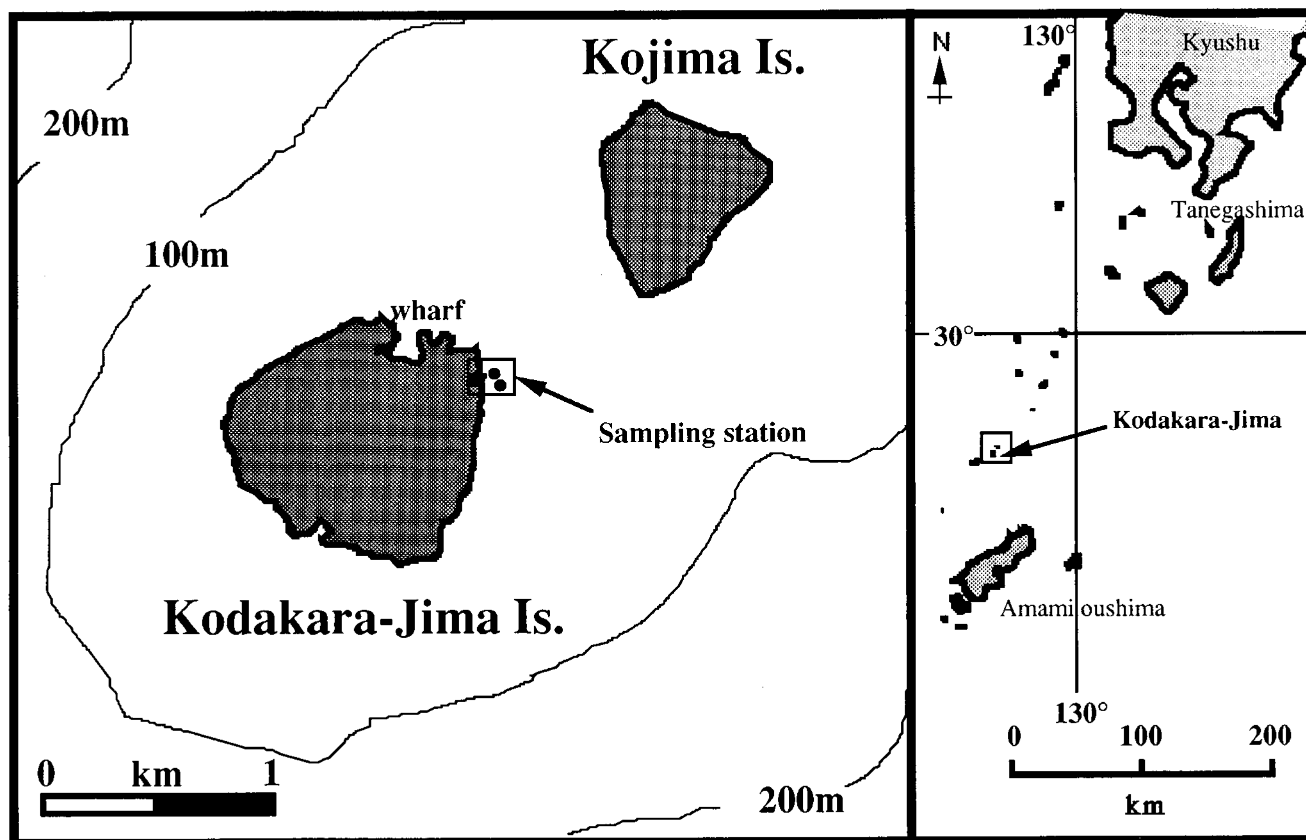

FIG. 1. Map of studied area and sampling station. The contour lines indicate the depth of the seawater layer. Is., Island.

total sulfur contents deposited in the sediments, samples were shaken vigorously, freed from most of the void seawater, dried at 105°C, and later analyzed by an EA1108 CHNS-O elemental analyzer (Carlo Erba Instruments, Rodano, Italy) equipped with a TCD detector, with nitrogen as the carrier gas at a flow rate of 100 ml · min^{-1} (oven temperature, 60°C; furnace temperature, 1,000°C; filament temperature, 190°C).

Contents of H_2S, SO_2, and CO in the gas were analyzed with gas analysis kits (Gastec, Yokohama, Japan). CO_2, H_2, and CH_4 were analyzed by gas chromatography as described previously (11).

Microbial populations. All procedures except preparation of media were performed in the ship's laboratory. Total microbial population densities in the seawater and sediment samples were estimated by the acridine orange direct-count method (13). The most-probable-number technique (8) was used to estimate the population densities of culturable anaerobic hyperthermophiles. The media were prepared by a Hungate procedure (16). The media used were SME medium (gas phase, H_2-CO_2 [80:20]; 200 kPa) (39) for hyperthermophilic S^0-dependent facultative autotrophs, NSW medium (gas phase, N_2; 100 kPa) supplemented with 0.2% yeast extract and 1% S^0 (21) for hyperthermophilic S^0-dependent heterotrophs, and BSM medium (gas phase, H_2-CO_2 [80:20]; 200 kPa) (26) for hyperthermophilic methanogens. The pHs of all media were adjusted to 7.0 with 1 N H_2SO_4.

Sediment samples were shaken well in an anaerobic chamber (ANX-1; Hirasawa Co. Ltd., Tokyo, Japan) before inoculation. One-milliliter aliquots of each of the suspended samples were diluted in triplicate in three different media with 10-fold dilutions (final volume, 10 ml) in the anaerobic chamber. After dilution into the first tubes, the samples were taken out of the chamber and diluted into the series of media (10 tubes each) by using 1-ml syringes (22-gauge, 1.25-in. [ca. 3.2-cm] needle) by the anaerobic technique of Balch and Wolfe (2). They were then incubated in a drying oven at 90°C. Growth in the tubes was monitored by turbidity or microscopic observation for 7 days after inoculation. Specific gravities and wet and dry weights of sediments were measured by standard methods (8).

Microscopy. Organisms in the samples were inspected with a Nikon phase-contrast light microscope. Methanogens with cofactor F_{420} were observed under an epifluoresence microscope (Nikon, Tokyo, Japan) equipped with a V-2A filter (exitation, 380 to 425 nm) and a BA450 filter (emission, 450 nm). Transmission electron microscopy (H-7000; Hitachi Co. Ltd., Tokyo, Japan) of negatively stained cells was carried out as described by Kurr et al. (26).

Chlorophyll. After filtration of 300-ml seawater samples through GF-75 (0.3-μm-pore-size) filters (Advantec, Tokyo, Japan), the GF-75 filters were soaked in 90% acetone for 6 h in a dark refrigerator to extract photosynthetic pigments. The A_{750}, A_{664}, A_{647}, and A_{630} of the extract were measured with a UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). The concentrations of chlorophylls *a*, *b*, and *c* were calculated with equations proposed by Jeffrey and Humphrey (24).

After filtration of 20 ml of void seawater from the sediment samples, the GF-75 filters were soaked in methanol-chloroform (1:1) for 30 min at 0°C. The extracted chlorophylls were separated with a high-performance liquid chromatograph (Tosoh Co. Ltd., Tokyo, Japan) equipped with TSKgel ODS-80Ts (15 cm by 4.6 mm [internal diameter], 5-μm particle size; Tosoh Co. Ltd.) (29) with a few modifications. The initial flow rate of 1.0 ml · min^{-1} was changed to 2.0 ml · min^{-1} at 9 min. The eluted pigments were identified by comparison of the retention times and by comparison of the absorption spectra with those of the pigments of *Chlorella vulgaris* NIES-227, *Pavlova lutheri* CS-23, and *Rhodobacter sphaeroides* ATCC 11166. Quantification of each pigment was carried out by the method of Mantoura and Llewellyn (28).

Nitrogen and phosphate. For the analyses of total nitrogen and total phosphate, samples of free seawater and void seawater from the sediments were used without filtration. Filtered (GF-75) samples were used for the analyses of NH_3-N, NO_2-N, NO_3-N and PO_4-P.

Total nitrogen was analyzed with a total-nitrogen automatic analyzer, TN-301P (Yanagimoto, Kyoto, Japan). Ammonium, nitrite, and nitrate were analyzed with an autoanalyzer type II (Bran & Luebbe, Norderstedt, Germany) by the phenate method intensified with sodium nitroprusside, a colorimetric method, and a cadmium reduction method, respectively (8). P_i was measured with the autoanalyzer type II by the ascorbic acid method (8). Total phosphate was analyzed in the same manner as P_i after digestion with persulfate in an autoclave (120°C, 30 min).

Organic carbon. Total organic carbon (TOC) and dissolved organic carbon (DOC) of both free seawater and void seawater in sediment samples, homogenized by ultrasonification (SONIFIER 250; Branson, Danbury, Conn.), were analyzed with a TOC 5000 TOC analyzer (Shimadzu). Samples for DOC analysis were passed through a 0.2-μm-pore-size filter (Nuclepore, Pleasanton, Calif.) before injection into the analyzer.

HAA and FAA. Void seawater from the sediment samples was passed through the GF/F filter and concentrated two to three times by lyophilization (final

volume, 0.5 ml) before analysis. After hydrolysis with 6 N HCl (110°C, 24 h), total amino acids, which include both hydrolyzable amino acids (HAA) and free amino acids (FAA), were analyzed with a JLC-300 amino acid analyzer (JEOL, Tokyo, Japan). FAA were analyzed after filtration (Nuclepore; 0.2 μm pore size) without prior hydrolysis. Concentrations of HAA were calculated by subtraction of the FAA concentrations from the total amino acid concentrations.

For the analysis of amino acids in filtered seawater, samples were concentrated as follows. A 100-ml seawater sample was passed through a glass column (internal diameter, 1 cm) containing granular activated charcoals (8-32 mesh, 2.5 g; Takeda Co. Ltd., Osaka, Japan) to prevent salting out. After the column was washed with distilled water, 5 ml of 0.1 N HCl was passed through it. The column was washed again with distilled water, and then amino acids were eluted with 30 ml of ethanol and concentrated 100 times in a rotary evaporator (final volume, 1 ml). Values for whole total amino acids for the concentrated samples were measured by FP-770 spectrofluorometry (excitation, 340 nm; emission, 455 nm) (JEOL) after hydrolysis (6 N HCl, 120°C, 12 h) and reaction with *o*-phthalaldehyde in potassium borate buffer (400 mM, pH 10.4) by the method of Robrish et al. (35). Whole FAA values were measured without hydrolysis.

Trace elements. Sediment samples (1 g [dry weight]) were digested by nitric acid-perchloric acid (8) before analysis. Concentrations of the trace elements (B, Na, Mg, Al, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Mo, Cd, and W) in the samples were measured with an ICPS-1000 inductively coupled sequential plasma spectrometer (Shimadzu).

RESULTS

Study area. While volcanic gas bubbles were being emitted from various hot spots on the sandy or rocky sea floor in an inlet of Kodakara-Jima Island, the compositions of the gases were all similar. The gas contained 82.2% ± 1.5% CO_2, 9.0% ± 1.0% H_2, 1.1% ± 0.1% CO, and 3.7% ± 2.8% unidentified gases. The most detailed study was undertaken at station 8, a sandy (1- to 3-mm-diameter particles) patch of sea floor located inside the inlet at a depth of 3 m with a calm tidal current. The flow rate of the volcanic gas emitted from a single spot (an approximately 5-mm-diameter outlet) was relatively low at this station, namely, 27.3 ml · min^{-1} · $spot^{-1}$. The concentration of dissolved hydrogen sulfide in void seawater at a depth of 10 cm in the sediments was 882 μM. The total sulfur content in void seawater extracts of the sediments was 19.6% ± 7.8% (wt/wt). The gas-evolving hot area was surrounded by an area with more moderate temperatures that was covered with white microbial mats (Fig. 2). Microscopic observation showed that these mats were composed of *Beggiatoa*-like filamentous bacteria ca. 7 to 18 μm wide, with intracellular sulfur particles (arrows in Fig. 2). No macroscopic invertebrate was found in the study area.

Hydrographic conditions and microbial communities. At station 8 the temperature of the seawater was 27°C at 100 cm above the sea floor and increased with depth in the sediments (80°C at 0 cm and 104°C at 30 cm from the sea floor surface) (Fig. 3a). The pH of the seawater at 100 cm above the sea floor was 7.89, and it was 7.24 at 10 cm. The pH of the void seawater in 0- to 30-cm sediments was 7.0 to 7.5. The salinity of the seawater (36.0‰) increased slightly (to 37.0‰) at a depth of 30 cm in the sediments (Fig. 3a). The total bacterial population in the seawater as determined by acridine orange direct counts was 3×10^6 to 5×10^6 cells · ml^{-1}, and that in the sediment was 8.6×10^6 cells · g of $sediment^{-1}$ at 0 cm and increased with depth in the sediments (1.2×10^7 cells · g of $sediment^{-1}$ at 10 cm and 1.7×10^8 cells · g of $sediment^{-1}$ at 30 cm from the sea floor surface) (Fig. 3b). The most-probable-number method showed that culturable hyperthermophilic S^0-dependent heterotrophs were almost absent in the seawater above the hot area but increased with increasing depth and temperature in the sediments (Fig. 3b). Their percentage also increased with depth in the sediments (0.5% at 0 cm, 1.5% at 10 cm, and 18% at 30 cm). Electron microscopy of an enriched culture of S^0-dependent heterotrophs showed that they closely resembled bacteria with unique fiber networks which had been isolated previously from this study site (Fig. 4) (11). A most-probable-number study showed no evidence for any hyperthermophilic methanogen in the seawater or sediments. However, under UV light excitation, a few weakly blue-fluorescent cocci were observed in the sediment samples of the hot area and on *Beggiatoa* filaments in the samples from the surrounding moderate-temperature area (data not shown). While hyperthermophilic S^0-dependent facultative autotrophs were not detected in the seawater, their density increased with depth in the sediments (approximately 3×10^2 cells · g of $sediment^{-1}$ at 30 cm) (Fig. 3b). Concentrations of chlorophyll *a* were 1.4 and 2.5 μg · $liter^{-1}$ in the seawater at 10 and 100 cm, respectively, above the sea floor and 34.4 μg · $liter^{-1}$ in the void seawater of surface sediments (Table 1). The ratios of chlorophyll *a* to

FIG. 2. Photomicrographs of *Beggiatoa*-like cells collected at the surface of the hydrothermal sediment. Arrows indicate intracellular sulfur particles. (a) Phase-contrast microscopy (bar, 100 μm); (b) bright-field microscopy (bar, 20 μm).

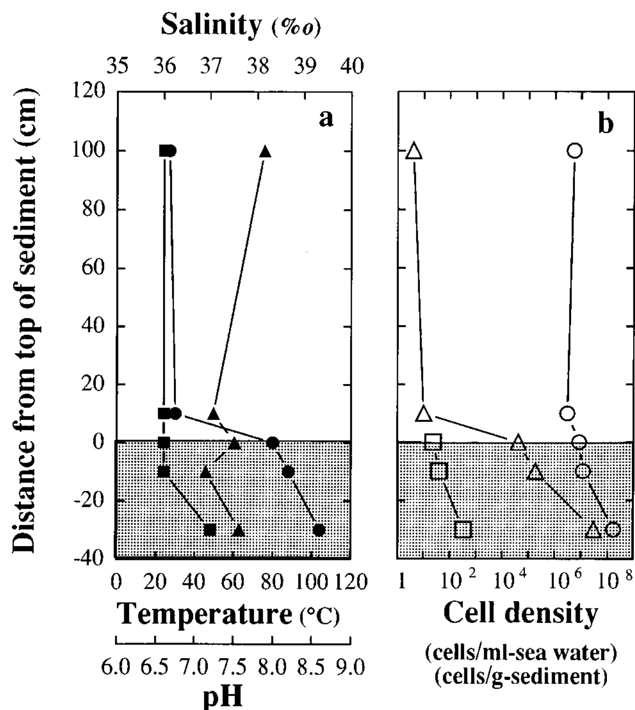

FIG. 3. Vertical profiles of hydrographic conditions and population densities of total microorganisms and hyperthermophiles at the hydrothermal sediment. Shaded areas indicate sediments, and unshaded areas indicate seawater. (a) Hydrographic conditions. Symbols: ●, temperature; ▲, pH; ■, salinity, (b) Microbial populations. Symbols: ○, total microorganisms (AODC); △, hyperthermophilic S^0-dependent heterotrophs; □, hyperthermophilic S^0-dependent autotrophs. Cell densities in sediments indicate cell densities in void seawater after multiplication by volume of void seawater (milliliters) and division by dry weight of sediment (grams).

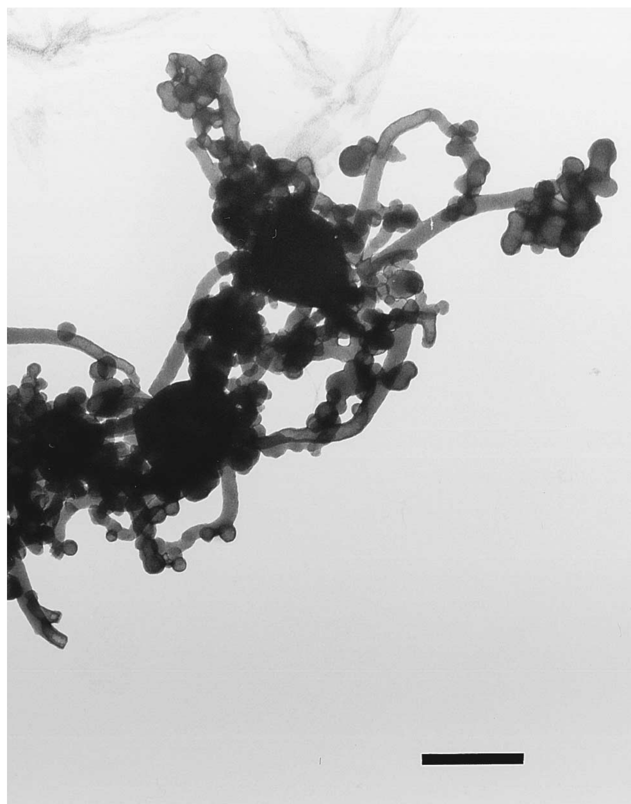

FIG. 4. Transmission electron microphotograph of negatively stained (uranyl acetate), enriched heterotrophic S^0-dependent hyperthermophiles grown in NSW medium supplemented with 0.2% yeast extract and 1% elemental sulfur. Bar, 1 μm.

chlorophyll *b* and of chlorophyll *a* to chlorophyll *c* were 2.3 and 0.8, respectively, in the seawater at 100 cm above the bottom and were 19.1 and 95.5, respectively, in the sediments. Bacteriochlorophylls were not detected in the seawater or sediments, indicating the absence of photosynthetic bacteria in these hydrothermal areas.

Vertical distribution of carbon, nitrogen, and phosphate. Vertical profiles indicated that nitrogen, phosphate, and organic carbon concentrations in the sediments (Fig. 5) were inversely proportional to the population densities of hyperthermophiles (Fig. 3b). Their concentrations decreased with increasing depth in the sediments. TOC and DOC concentrations in the seawater were 2 to 4 and 1 to 2 mg · liter^{-1}, respectively (Fig. 5a). TOC and DOC concentrations in the void seawater of surface sediments were 240 and 59.3 mg · liter^{-1}, respectively (Table 2). This indicates that 75.3% of the TOC was particulate organic carbon in void seawater of the surface sediments. Percentages of TOC in void seawater at 10 and 30 cm in the sediments were 23.1 and 22.7%, respectively, of that in the surface sediments. Percentages of particulate organic carbon in the TOC at depths of 10 and 30 cm in the sediments were 40.6 and 38.3%, respectively.

The concentrations of total nitrogen, NH_3-N, NO_3-N, and NO_2-N in the seawater at 100 cm above the sea bottom were 0.2, 0.02, 0.03, and 0.01 mg · liter^{-1}, respectively. The concentration of total nitrogen in void seawater of the surface sediments was 11.3 mg · liter^{-1}, and it contained 47.1% inorganic nitrogen (43.6% NH_3-N, 3.4% NO_3-N, and a trace amount of NO_2-N) (Fig. 5b). The total nitrogen concentration in void seawater at a depth of 30 cm in the sediments was 23% lower than that in the surface sediments.

Eighty-five percent of the total phosphate in void seawater of the surface sediments was P_i. Concentrations of both total phosphate and P_i in void seawater decreased with the depth in the sediments.

Amino acids. The concentrations of whole HAA and FAA in the seawater column were approximately 0.50 and 0.02 mg · liter^{-1}, respectively. HAA and FAA concentrations in void seawater of the surface sediments were 17.7 and 21.2 mg · liter^{-1}, respectively (Table 3), which constituted 10.1 and 13.2% of the DOC, respectively (Table 2). While the contribution of the carbon contents of whole HAA to DOC did not change with depth in the sediments, the concentration of the carbon contents of whole FAA and its percentage in DOC decreased with depth, e.g., 1.8 mg · liter^{-1} (5.5% of DOC) at 10 cm and 0.9 mg · liter^{-1} (2.7%) at 30 cm. In the total amino acids in the surface sediments, the individual amino acids were

TABLE 1. Chlorophyll concentrations showing change with depth

Seawater depth (cm)[a]	Concn (μg/liter) of chlorophyll:			Ratio of chlorophyll concn	
	a	*b*	*c*	*a/b*	*a/c*
100	1.4	0.6	1.8	2.3	0.8
10	2.5	0.2	0.1	12.5	25.0
0	34.4	1.8	0.36	19.1	95.5

[a] Depth above sediment surface (0 cm).

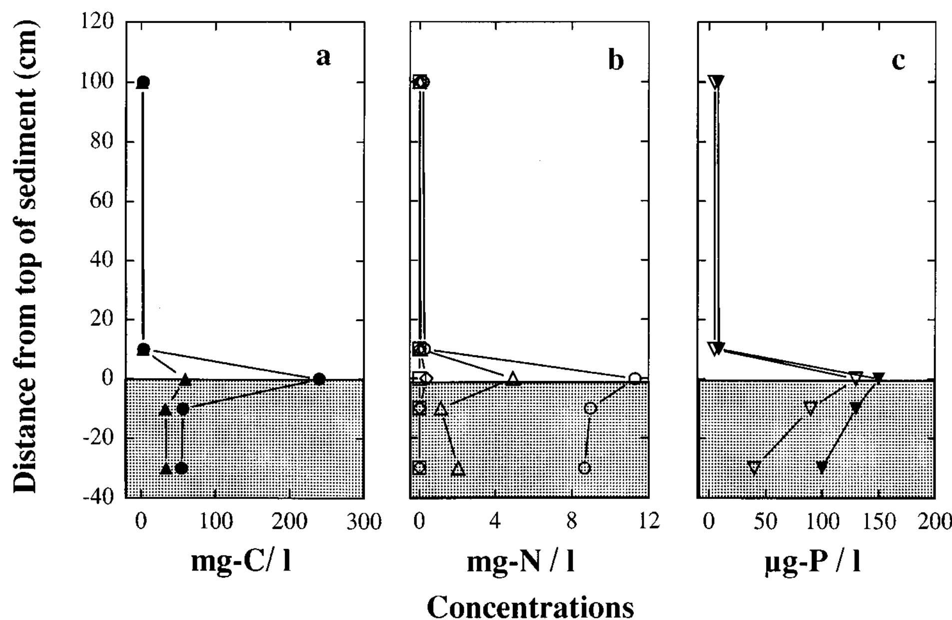

FIG. 5. Vertical profiles of carbon, nitrogen, and phosphate in hydrothermally active sediments. Concentrations in sediments indicate concentrations in void seawater. (a) Concentrations of TOC (●) and DOC (▲); (b) concentrations of total nitrogen (○), NH_3-N (△), NO_2-N (□), and NO_3-N (◇); (c) concentrations of total phosphate (▼) and PO_4-P (▽). For additional explanations, see the legend to Fig. 3.

in the following order of abundance (numbers in parentheses indicate concentrations in milligrams per liter): taurine (12.1) > Asn (6.8) > Ala (4.7) > Glu (3.7) > Gly (3.4) > Ile (2.0) > Leu (1.7) > Val (1.6) > Asp (1.5) > Ser (0.8) > Thr (0.7) (Table 3). Pro, Cys, Met, Tyr, Phe, Trp, Lys, His, and Arg were not detected in any of the sediment samples (Table 3).

Trace elements. The major trace elements in the sediments were Na, Mg, Al, K, Ca, and Fe (Table 4). The trace elements included in the culture medium for hyperthermophilic S^0-dependent heterotrophs, viz., B, Mn, Co, Ni, Cu, Zn, Sr, Mo, and W (21), were also detected in the sediments.

DISCUSSION

Station 8 was suitable for the study of the ecosystem of hyperthermophiles because of calm tidal currents and shallow depths due to the following features: first, the station was located inside an inlet, and second, there was little mixing by turbulence due to gas emission. In contrast, at an oceanic shallow geothermally active zone of north Ogasawara, Japan (25°27′75″N, 141°14′40″E; depth, 26 m), there is a strong tidal flow and a gas production rate approximately 400 times higher (12 liters · min^{-1} · $spot^{-1}$) than that observed at station 8 (unpublished data). Therefore, the habitat at north Ogasawara was disturbed by turbulent hydrothermal fluid and seawater mixing. In the case of deep-sea hydrothermal vents, flow rates

TABLE 2. Organic carbon balance in void seawater in the hydrothermal sediments

Carbon[a]	mg of C/liter (%) at the following depth[b]:		
	0 cm	10 cm	30 cm
TOC	240.1	55.4	54.6
POC	180.8 (75.3)	22.5 (40.6)	20.9 (38.3)
DOC	59.3 (24.7)	32.9 (59.4)	33.7 (61.7)
HAA-C	6.00 (10.1)	4.7 (14.3)	4.0 (12.0)
FAA-C	7.8 (13.2)	1.8 (5.5)	0.9 (2.7)

[a] POC, particulate organic carbon (TOC − DOC); HAA-C, carbon content of whole HAA; FAA-C, carbon content of whole FAA.

[b] Depth in sediments (0 cm, surface of sediments). For particulate organic carbon and DOC, percentages are those in TOC; for the carbon contents of whole HAA and FAA, percentages are those in DOC.

TABLE 3. FAA and peptide concentrations in void seawater of the hydrothermal sediments

Amino acid	Concn (mg/liter) at the following depth[a]:					
	0 cm		10 cm		30 cm	
	FAA	HAA	FAA	HAA	FAA	HAA
Taurine	5.6	6.5	2.4	1.6	0.8	1.0
Asp	1.5	ND[b]	0.2	ND	0.2	ND
Thr	0.7	ND	ND	ND	ND	ND
Ser	0.8	ND	0.2	ND	ND	ND
Asn	ND	6.8	ND	3.2	ND	3.5
Glu	3.7	ND	0.8	0.1	0.6	0.4
Gln	ND	ND	ND	1.0	ND	1.0
Pro	ND	ND	ND	ND	ND	ND
Gly	3.4	ND	1.5	ND	0.4	ND
Ala	3.7	1.0	0.9	0.9	0.4	0.4
Val	0.8	0.8	ND	1.7	ND	1.7
Cys	ND	ND	ND	ND	ND	ND
Met	ND	ND	ND	ND	ND	ND
Ile	1.0	1.0	ND	0.5	ND	0.7
Leu	ND	1.7	ND	2.5	ND	1.0
Tyr	ND	ND	ND	ND	ND	ND
Phe	ND	ND	ND	ND	ND	ND
Trp	ND	ND	ND	ND	ND	ND
Lys	ND	ND	ND	ND	ND	ND
His	ND	ND	ND	ND	ND	ND
Arg	ND	ND	ND	ND	ND	ND
Total	21.2	17.7	6.0	11.3	2.4	9.9

[a] Depth in sediments (0 cm, surface of sediments).

[b] ND, not detected (<0.1 mg/liter)

TABLE 4. Concentrations of trace elements in the hydrothermal sediments

Element	Concn (mg/kg)[a]
Na	8,700 ± 1,600
Mg	14,800 ± 4,700
Al	10,300 ± 6,700
K	1,700 ± 100
Ca	8,900 ± 3,500
Fe	14,700 ± 8,100
B	107.0 ± 25.6
V	38.3 ± 4.8
Mn	728.0 ± 34.1
Co	132.4 ± 1.7
Ni	13.3 ± 2.9
Cu	232.0 ± 2.6
Zn	96.9 ± 47.5
Se	222.0 ± 94.1
Sr	118.4 ± 68.2
Mo	35.1 ± 4.4
Cd	16.0 ± 1.8
W	791.0 ± 54.4

[a] Mean ± standard deviation ($n = 3$).

for the hydrothermal fluid were reported to be 200 $cm \cdot s^{-1}$ at hot vents and 1 to 2 $cm \cdot s^{-1}$ at warm vents (19).

The composition of evolving volcanic gas was quite similar to that in the deep-sea Okinawa backarc basin (36), which was located on the same volcanic belt, approximately 280 km south of this study site at a depth of 1,300 to 1,500 m. Concentrations of metals in the sediments were similar to those of an East Pacific Rise vent (4). These features indicate that the hot fluid in the present study area is possibly derived from deep groundwater passing through a magmatic chamber (6).

In the seawater at station 8, only a few hyperthermophiles were detected at 10 cm, and none were detected at 100 cm above the active hydrothermal spots. In contrast, after violent volcanic eruptions at the Macdonald seamount, hyperthermophilic archaea were detected at densities of 10^4 to 10^6 $cells \cdot ml^{-1}$ in marine surface waters 0.5 km away from the active zone, arriving with the advection of a hydrothermal plume (15). The differences in their distributions and densities may depend not only on the magnitudes of the eruptions but also on the ability of the hyperthermophiles to survive in ordinary marine environments.

In these hydrothermal sediments, hyperthermophilic S^0-dependent heterotrophs are numerically dominant. The dominant hyperthermophiles in our enriched cultures had tubular networks (Fig. 4) and were similar to the *Thermoccocus* spp. isolated from this study site previously (11). Although the high population density of S^0-dependent heterotrophs in the sediments was comparable to the maximum cell density of a batch culture of *Thermococcus* spp. grown in vitro, concentrations of FAA in the sediments were much lower than those required for growth of the isolates in the culture medium; e.g., *Thermococcus* spp. require at least 1 g of amino acid mixture $\cdot$ $liter^{-1}$ to reach a density of 10^8 $cells \cdot ml^{-1}$ (11). At their habitats, the concentration of the carbon contents of whole FAA and its percentage of DOC decreased with the depth of sediment. In addition, amino acids essential for the growth of *Thermococcus* spp., notably Thr, Leu, Ile, Val, Met, Phe, Tyr, His, Lys, and Arg (11), were not detected among the FAA in the deeper sediments. These results indicate that the lower FAA concentration is probably due to consumption by dense populations of the S^0-dependent heterotrophs. Consequently, transport of the decomposed organic matter, derived mostly from the primary producers, and its deposition in the sediments would be important for the survival of hyperthermophilic heterotrophs in high-temperature habitats. The minimum amounts of individual amino acids may be supplied continuously in the habitats by seawater circulation and fluctuating hydrothermal flow around the eruption sites (10).

The presence of strictly anaerobic methanogens or S^0-dependent autotrophs in these high-temperature biotopes supports the hypothesis that chemolithotrophic hyperthermophiles produce organic matter (15, 38). While hyperthermophilic methanogens were not detected in this study, a few cells with blue-green fluorescence were observed, whose fluorescence might derive from F_{420} of methanogens (1, 26) or sulfate-reducing archaea (37). The percentage of culturable hyperthermophiles in the total bacterial population of the sediments was less than 20%, although it increased with depth. These results indicate that nonthermophilic marine bacteria and plankton may be deposited at surface sediments. In addition, unknown or unculturable hyperthermophiles may contribute to the total bacterial population; the existence of unknown rRNA genes belonging to crenarchaeal species was demonstrated at a hot spring in Yellowstone National Park in the United States (3).

The abundant particulate organic substance at the surface of the sediment might derive mostly from white microbial mats growing around the volcanic gas eruption sites. It has been reported that at deep-sea vents H_2S is consumed by chemolithotrophic sulfur-oxidizing bacteria, *Beggiatoa* spp. (31, 34). The surface of the sediments at the present study site also seems to provide a suitable habitat for *Beggiatoa* spp., because hydrogen sulfide in the sediment was at a concentration (0.88 mM) comparable to that at deep-sea hot vents (0.5 to 10 mM) (19). On the other hand, the predominance of chlorophyll *a* near the hot spot indicates the presence of phytoplankton (27, 32). In similar environmental conditions, the presence not only of chemolithotrophic sulfur-oxidizing bacteria but also of cyanobacteria and picophytoplankton has been demonstrated in a shallow submarine hydrothermal area in the Porto di Levante of Vulcano Island, Italy (9). These chemotrophic and phototrophic autotrophs may contribute to the production of abundant organic substances, especially proteins and amino acids, at the surface of the sediment. At that point, the products of *Beggiatoa* sp. decay would be a suitable nutritional source for the S^0-dependent heterotrophs, since they could provide both protein and sulfur. The particulate proteins in the sediments possibly are degraded and consumed by dense populations of the S^0-dependent heterotrophs, because such heterotrophs isolated from the present study site exhibit protease activities and can grow by using proteins supplied in the medium (11, 30).

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