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Chemolithotrophic Sulfur-Oxidizing Bacteria from the Galapagos Rift Hydrothermal Vents†

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Three distinct physiological types of sulfur-oxidizing bacteria were enriched and isolated from samples collected at several deep-sea hydrothermal vents (2,550 m) of the Galapagos Rift ocean floor spreading center. Twelve strains of the obligately chemolithotrophic genus *Thiomicrospira* were obtained from venting water and from microbial mats covering surfaces in the immediate vicinity of the vents. From these and other sources two types of obligately heterotrophic sulfur oxidizers were repeatedly isolated that presumably oxidized thiosulfate either to sulfate (acid producing; 9 strains) or to polythionates (base producing; 74 strains). The former were thiobacilli-like, exhibiting a thiosulfate-stimulated increase in growth and CO_2 incorporation, whereas the latter were similar to previously encountered pseudomonad-like heterotrophs. The presence of chemolithotrophic sulfur-oxidizing bacteria in the sulfide-containing hydrothermal water supports the hypothesis that chemosynthesis provides a substantial primary food source for the rich populations of invertebrates found in the immediate vicinity of the vents.

Chemoautotrophic, sulfur-oxidizing bacteria are characteristically found in environments of low redox potential in which reduced sulfur compounds and molecular oxygen occur simultaneously (for recent reviews see references 15 and 17). In the anoxic muds of marine estuaries and coastal sediments the anaerobic, heterotrophic metabolism of sulfate-reducing bacteria is responsible for most of the production of hydrogen sulfide (H_2S). As the original source of reduced sulfur compounds, H_2S supports abundant populations of sulfur-oxidizing bacteria at the oxic-anoxic interface. Sulfate reduction appears to be far less prevalent in the relatively oxic, organic-poor deep-sea sediments and the open-ocean water column (4, 29). Thus, in much of the offshore waters reduced inorganic sulfur exists at low or undetectable concentrations, and the presence of chemoautotrophic, sulfur-oxidizing bacteria has been correspondingly difficult to demonstrate by either isolation or activity (1, 12, 34).

Recently discovered hydrothermal springs along ocean floor spreading centers (e.g., the Galapagos Rift) have been found to release geothermally produced H_2S and other reduced inorganic compounds into the surrounding waters (5, 6). Associated with these vents were unusually dense invertebrate communities (8, 24). Because it is unlikely that, at a depth of over 2,500 m, these communities receive a significant and sustainable input of organic carbon from surface water photosynthetic primary production, they have been proposed to be supported by the chemosynthetic activity of bacteria oxidizing reduced inorganic compounds issuing from the vents (5, 13). Direct microscopic observation (5, 13) and nucleotide analyses (16) of vent water have indicated the presence of high concentrations of bacterial biomass that may reflect substantial bacterial production associated with the vents. Microbial growth is most likely occurring on the large surface areas within the subsurface vent system. Geological and geochemical evidence (5, 22, 26) indicates that oxygenated seawater percolates through the highly permeable pillow lava, mixing at certain depths with the upwelling reduced vent water.

The high concentration of H_2S, as compared to other inorganic compounds available for chemosynthesis (H_2, NH_3, NO_2^-, Fe^{2+}, and possibly Mn^{2+}) (6, 7, 21), suggested that H_2S is the predominant energy source for chemosynthesis. This report describes the results of efforts to obtain sulfur-oxidizing bacteria from enrichment cultures inoculated with samples of vent water and surface materials collected near the vents.

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MATERIALS AND METHODS

Sample collection. The hydrothermal vents, located at depths of 2,500 to 2,600 m in a region of the central Pacific Ocean approximately 640 km west of Ecuador and 330 km northeast of the Galapagos Islands, were visited in January and again in November to December 1979. With the aid of the deep-sea research submersible *ALVIN*, vent water was collected with sterile Niskin samplers as well as with an in situ pumping system equipped with membrane filters (0.45-μm pore size; Millipore Corp.). Mussels, clams, and pieces of lava from the vicinity of the vents were brought to the surface in closed containers. Bits of surface materials, especially from the soft periostracum layer of mussels, were used as inocula for enrichment cultures. Pieces of glass slides and polycarbonate filters deposited in one of the vents as a surface growth experiment over the 10-month interval between cruises were also used as inocula.

Enrichments. Enrichments were made either from freshly collected material aboard the research vessel or from material that had been stored at 4°C for as long as 1 month. The following previously described thiosulfate-based media vary with respect to the use of natural or artificial seawater and the concentrations of other ingredients: TB (34); AG (1); K (19); and, for denitrifying thiobacilli, BL (2). S represents a modified medium of Postgate (from reference 34) for oxidizers of elemental sulfur. NF represents TB medium lacking the combined nitrogen (NH_4Cl) but containing 12 μM Na_2MoO_4 in a 0.2% slush agar. TAC represents an acetate-catalase medium used by Strohl and Larkin (30) for the enrichment of freshwater *Beggiatoa*, modified by the addition of 520 mM NaCl, 20 mM thiosulfate, and 1 ml of trace elements solution (36) per liter. Enrichments were incubated at either 4 or 21°C, and phenol red (0.0005%) was used as a pH indicator in most media.

Sulfide gradients were set up in test tubes (15 by 120 mm) containing a 4-ml 1.5% agar plug in the bottom with 0.005 to 0.05% neutralized Na_2S (pH 8.0). The plug was overlaid with 10 ml of TB (without thiosulfate) in 0.2% agar, and the tube was sealed with a rubber stopper to allow establishment of the gradient. Within 10 h the inoculum was introduced by insertion midway into the tube.

Nutritional characterization. Heterotrophic strains were examined by replicate plating for the ability to grow on one of 17 compounds as the sole source of carbon and energy. Media were solidified with 1.5% Difco Noble agar. Plates were incubated at 22°C, and after 3 and 12 days, growth on the carbon source was ascertained by comparison with control (no added carbon) plates (3). The basal medium (ASW) consisted of Lyman and Fleming's formulation (25) for artificial seawater (400 mM NaCl, 57 mM $MgCl_2 \cdot 6H_2O$, 28 mM Na_2SO_4, 10 mM $CaCl_2$, 8.5 mM KCl, and 2.4 mM $NaHCO_3$, supplemented with 20 mM NH_4Cl, 1 ml of trace elements solution (36) per liter, and 0.05% potassium phosphate buffer (pH 7.1). Growth at 4, 22, and 38°C was determined on solidified medium (1.5% agar) and at 55°C in liquid medium containing 0.3% Difco peptone in ASW. Formation of gas during anaerobic incubation with 30 mM KNO_3 was observed in 0.7% agar stab tubes containing ASW with 10 mM thiosulfate. After inoculation, the medium was covered with an agar plug and examined weekly over a period of 1 month for the presence of gas bubbles. Gas formation during glucose fermentation was determined similarly in ASW containing 0.1% glucose as the sole carbon and energy source and prepared by anaerobic techniques (11).

All isolates requiring inorganic sulfur were tested in ASW for the ability to use thiosulfate (12 mM), sulfide (2 mM), sulfite (3 mM), thiocyanate (2 mM), elemental sulfur (0.05%, wt/vol), or tetrathionate (10 mM) as the sole source of energy. Tetrathionate was synthesized by the method of Trudinger, which reportedly produces a 99 to 100% pure product as described by Roy and Trudinger (27). The ASW thiosulfate medium was used to determine growth within a pH range of 4.5 to 8.5 as adjusted by monopotassium-dipotassium phosphate buffer with a final concentration of 0.05% (wt/vol).

Growth studies. Strains of obligate lithotrophs and heterotrophs were pregrown in ASW-thiosulfate (T-ASW) medium and in T-ASW-yeast extract (TY-ASW) medium, respectively. From these cultures three media were inoculated to initial cell densities of 10^6 cells per ml: ASW, T-ASW (10 mM thiosulfate), and Y-ASW (0.01% [wt/vol] yeast extract). Growth was measured by epifluorescence microscopy (9) and is described as the ratio between maximum cell density in either T-ASW or Y-ASW and that in ASW, the unsupplemented control.

A more specific study on strain NF-18 was made of the effect of thiosulfate on growth in the presence of higher concentrations of organic substrates (0.05, 0.1, and 0.2% [wt/vol] yeast extract or glucose) in either ASW or T-ASW. Optical density was monitored at 600 nm, and thiosulfate concentration was determined by the method of Sorbo (28).

$^{14}CO_2$ incorporation studies. Cultures were pregrown as above. Log-phase cell suspensions were centrifuged at 10,000 × *g* for 10 min at 4°C, and the resulting pellet was suspended to a cell density of approximately 10^5 cells per ml in 340 ml of ASW containing 10 mM piperazine-*N*,*N'*-bis(2-ethanesulfonic acid) (PIPES) buffer, pH 7.0 (50 mM PIPES was used for obligate chemolithotrophs). Cells were preincubated at room temperature for 1 h, after which the cell density was determined by epifluorescence microscopy. Fifty microcuries of $NaH^{14}CO_3$ was added to the culture, and 40 ml was dispensed to each of eight 50-ml flasks (specific activity, approximately 1,500 cpm/μg of $NaHCO_3$). Duplicate flasks received either 10 mM thiosulfate, 0.01% yeast extract, both, or no additions. At five intervals over a 23-h period 7 ml was removed from each flask and filtered through a 22-mm-diameter membrane filter (Amicon Corp.; 0.2-μm pore size). Filters were washed twice with 5 to 10 ml of seawater and placed in a desiccator with fuming HCl for 15 to 20 min. Filters were transferred to vials and aired for 1 h before adding 10 ml of Bray solution for liquid scintillation counting. Corrections for quenching were made using the channels ratio method. At the final sampling (23 h) the cell density was determined by epifluorescence microscopy. Chemical analyses of total cell carbon in cultures were performed

on duplicate samples retained on precombusted glass-fiber filters (GF/F) with a Perkin-Elmer 240 elemental analyzer.

RESULTS

Successful enrichments were indicated by a significant change in medium pH or the precipitation of particulate sulfur (or both). From the various media 95 sulfur-oxidizing strains were isolated (Table 1). Although the enrichment conditions (sulfur source, initial pH, temperature, oxygen availability, etc.) differed to facilitate the isolation of a number of metabolic types of sulfur-oxidizers, all of these strains grew on a thiosulfate-agar medium (TB). This medium was thus used for purification and storage of the strains. In addition to sulfur-oxidizing strains, many of the enrichments yielded heterotrophic bacteria that did not oxidize reduced sulfur, but instead were capable of efficient growth on organic contaminants present in the media. These non-chemolithotrophic strains are not dealt with further in this report.

The 95 sulfur-oxidizing strains included a group of 12 obligate chemolithotrophs that required a growth medium containing a reduced sulfur source such as thiosulfate, tetrathionate, elemental sulfur, or sulfide. Colonies of these strains formed a white precipitate of elemental sulfur on TB agar medium and reduced the pH to below 5.0 even when the medium was supplimented with 50 mM PIPES buffer. Scanning electron microscopic examination of the strains showed comma- to spiral-shaped bacteria that were 0.3 to 0.4 μm wide and 0.7 to 2.0 μm long (Fig. 1A). When grown in soft agar overlay plates as described by Kuenen and Veldkamp (19), they exhibited the distinctive colony size and appearance of *Thiomicrospira pelophila* (Fig. 1B) and were physiologically indistinguishable from previously described strains of this genus (Table 2).

The remaining 83 strains grew heterotrophically in media containing peptone or yeast extract, with or without thiosulfate. On the basis of 28 traits these strains could be placed into similarity groups, each comprising strains differing by no more than one criterion. When grown on a thiosulfate-containing medium these groups could be divided into acid producers, which formed clear colonies (9 strains), and base producers, which precipitated elemental sulfur (74 strains). The characteristics of 61 of these 83 strains, comprising nine groups of base- or acid-producing isolates, are summarized in Table 3.

Representatives of these groups of sulfur-oxidizing heterotrophs were examined to determine whether the presence of thiosulfate resulted in increased growth and an enhanced incorporation of CO_2 into cell material. Three strains of *Thiomicrospira* (L-12, RTPMB, and TB-49) isolated from the vents, and *Thiobacillus* sp. (strain SS-T) obtained from a local salt marsh were included for comparison. The heterotrophic strains were inoculated at a concentration of about 10^5 cells per ml and were generally capable of growing to a maximum cell density of about 5×10^6 cells per ml on the contaminating material in the mineral salts medium (ASW). Although the addition of 0.01% yeast extract to ASW increased the growth of all the heterotrophic strains by 100- to 350-fold, yeast extract did not produce a significant increase in the growth of the obligately lithotrophic *Thiomicrospira* and *Thiobacillus* strains (Table 4). The addition of 10 mM thiosulfate to ASW medium had a small inhibitory effect on the growth of the base-producing heterotrophs. In contrast, both the obligate lithotrophs and the acid-producing heterotrophs had significantly increased growth in the presence of thiosulfate (Table 4).

The incorporation of CO_2 was stimulated by thiosulfate in the same strains that exhibited increased growth with thiosulfate addition. Base-producing strains such as TB5.5-9 incorporated only a small amount of CO_2 during cell growth in either ASW or T-ASW (Fig. 2). In both the presence and absence of thiosulfate the amount of CO_2 fixed per new cell formed was less than 3% of the total cell carbon. In contrast, in the acid-producing strains such as NF-18, thiosulfate caused a distinct stimulation of CO_2 incorporation (Fig. 2). Similarly, cultures of NF-18 grown in T-ASW exhibited a 25-fold greater incorporation of CO_2 per cell produced than non-lithotrophically grown cells (Y-ASW, Table 4).

TABLE 1. *Inocula and enrichment media used for isolating sulfur-oxidizing bacteria*

Inocula[a]	Media[b]	No. of strains isolated
F, M, MS, P, SB, VW	TB (pH 7.2)	39
F, VW	TB (pH 5.5)	8
F, M, MS, VW	AG	7
VW	K	14
VW	K plus 0.01% yeast extract	2
B, GS, VW	TAC	2
F, VW	BL	0
B, F, GS, M, MS, VW	NF	6
F, M, MS, VW	S	0
B, F, GS, MS, P, SB, VW	Sulfide gradient	17

[a] Abbreviations: B, filamentous material from settling panels; F, filter from in situ water-pumping system; GS, glass slide incubated 10 months in situ; M, mussel shell; MS, mussel stomach contents; P, mussel periostracum; SB, slide box scraping; VW, vent water sample.
[b] See text for media designations.

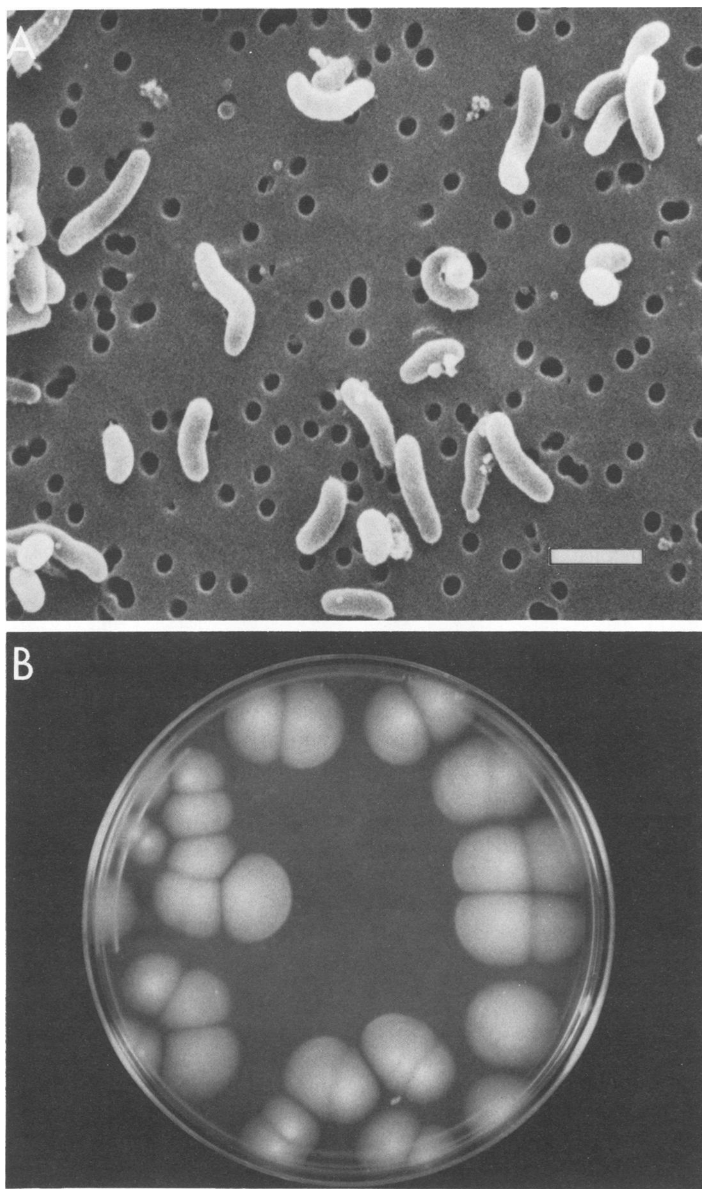

FIG. 1. *(A) Scanning electron micrograph of a pure culture of the vent isolate Thiomicrospira strain L-12 on a Nucleopore membrane filter (0.2-µm pore size). Bar, 1 µm. (B) Colonies of strain L-12 growing on 1.5% agar TB medium overlaid with 0.8% agar TB medium (see reference 19 for comparison).*

However, this increase did not occur when 0.01% yeast extract was added with thiosulfate (TY-ASW). This result was most dramatic in strain NF-18, in which we examined the effect of organic substrates on thiosulfate-stimulated growth in more detail (Table 5). As little as 0.05% of glucose or yeast extract eliminated the growth advantage due to thiosulfate oxidation that was demonstrated in Table 4.

In the presence of thiosulfate the three *Thiomicrospira* strains, as expected, exhibited an increase in growth that appeared to be limited only by their acidification of the medium (Table 4). The *Thiobacillus* strain SS-T grew to a higher cell density than the *Thiomicrospira* because of its greater pH tolerance. In all four of the obligate lithotrophs CO_2 incorporation required thiosulfate, and the amount fixed was equivalent to over 90% of the cell carbon produced. In contrast to the acid-producing heter-

TABLE 2. *Characteristics of obligate chemolithotrophic vent isolates and T. pelophila*[a]

Strain	Morphology	pH range for growth	S chemolithotrophy	Oxidation of: Sulfide	Sulfur	Tetrathionate	Thiosulfate	Sulfite	Thiocyanate	S° formation from excess thiosulfate	Main carbon source	Na^+ requirement	Gas from NO_3^- anaerobically	O_2 preference	mol% of G + C[b]
Galapagos strains	Motile; comma to spiral shape; 0.3 by 2 to 3 μm	5.5–8.5	Obligate	+	+	+	+	−	−	+	CO_2	+	−	Microaerophilic	44.4
T. pelophila	Motile; comma to spiral shape; 0.2 by 1 to 4 μm	5.0–8.5	Obligate	+	+	+	+	NR[c]	NR	+	CO_2	+	NR	Microaerophilic	48

[a] Data for *T. pelophila* are from reference 19.
[b] Mole percent of guanine plus cytosine, determined in only one Galapagos strain, L-12.
[c] NR, Not reported.

TABLE 3. *Similarity groupings of heterotrophic, thiosulfate-oxidizing isolates*

Group	No. of strains	Representative strain	pH change in TB[a]	Growth on the following as sole carbon source: Aspartate	Glutamate	Acetate	Pyruvate	Lactate	Succinate	Citrate	Gluconate	Glycerol	Ribose	Xylose	Glucose	Fructose	Galactose	Maltose	Sucrose	Lactose	Growth on peptone at: 4°C	22°C	38°C	55°C	Fluorescent colony	Fermentation of glucose	Gas formation anaerobically on: Glucose	NO_3^- and $S_2O_3^{2-}$	Cell size (μm)	Motility
I	18	AG-25	B	−	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+	2 by 0.8	+
II	13	K-12	B	+	+	+	−	+	+	+	+	+	−	−	+	−	−	−	−	−	−	+	−	−	+	ND[b]	ND	+	1.5 by 0.8	+
III	13	TB-A	B	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	−	−	+	−	+	1.5 by 1	+
IV	8	TB5.5-9	B	+	+	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	−	−	−	−	+	2 by 0.8	−
V	3	NF-13	B	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	−	−	−	+	2 by 0.7	+
VI	3	AG-33	A	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	−	+	+	−	−	−	−	+	2 by 1	−
VII	1	TB-66	A	+	+	+	+	+	+	−	−	+	−	−	+	+	−	−	−	−	+	+	−	−	−	−	−	−	2 by 1	−
VIII	1	NF-18	A	+	ND	+	+	+	+	+	+	+	+	ND	+	+	+	−	+	−	−	+	+	−	−	−	−	ND	1.5 by 1	+
IX	1	RTRG	A	−	ND	−	+	−	−	−	−	−	−	ND	−	−	−	−	−	−	−	+	+	−	−	ND	ND	−	1.5 by 1	−

[a] A, Acidic; B, basic.
[b] ND, No data.

TABLE 4. *Effect of thiosulfate and yeast extract on growth and CO_2 incorporation by representative isolates*

Strain	Final pH in T-ASW[a]	Growth[b] in:		μg of HCO_3^- fixed/10^7 cells produced in:		
		Y-ASW	T-ASW	Y-ASW	T-ASW	TY-ASW
Heterotrophs:						
AG-25	8.4	351	0.4	0.1	IG[c]	0.1
NF-13	8.8	115	0.7	0.2	IG	0.2
TB5.5-9	8.7	245	0.6	0.1	0.1	0.1
TB-A	7.9	ND[d]	ND	0.1	0.1	0.1
K-12	7.8	ND	ND	0.0	IG	0.1
NF-18	6.3	203	12	0.1	2.5	0.1
AG-33	6.5	218	7	0.2	0.6	0.2
TB-66	5.7	106	4	0.3	0.6	0.2
RTRG	6.2	ND	6	0.1	0.7	0.2
Lithotrophs:						
L-12	4.7	0.7	67	IG	3.1	3.2
RTPMB	4.7	0.6	56	IG	2.7	3.1
TB-49	4.7	1.4	86	IG	6.1	3.7
SS-T	3.9	2.0	199	IG	3.1	3.6

[a] Initial pH was 7.0.
[b] Ratio of maximum cell density (as determined by epifluorescence direct counts) over that in the unsupplemented control.
[c] IG, Insufficient growth for determination.
[d] ND, No data.

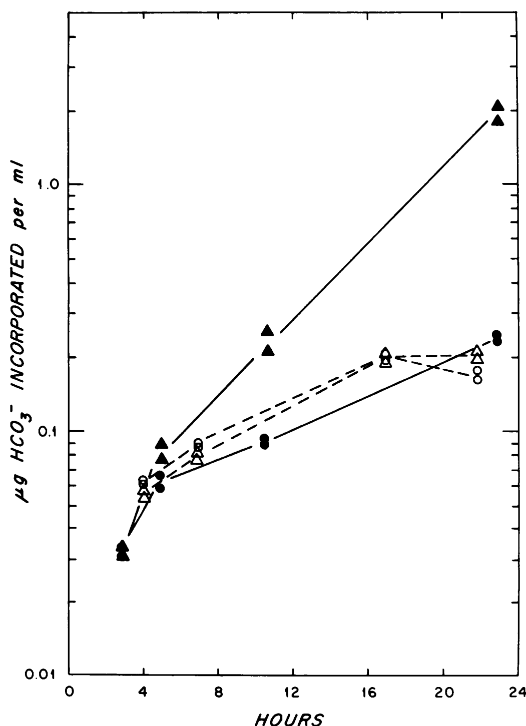

FIG. 2. *Incorporation of [^{14}C]bicarbonate by cultures of strains NF-18 (closed symbols) and TB5.5-9 (open symbols) during growth. Cells were inoculated into ASW (●, ○) or ASW plus 10 mM thiosulfate (▲, △).*

TABLE 5. *Effect of thiosulfate on growth of NF-18 in organic media*

Additions to ASW	Maximum growth (optical density at 660 nm)	% of thiosulfate utilized
0.05% Yeast extract	0.20	
0.1% Yeast extract	0.38	
0.2% Yeast extract	0.66	
0.05% Yeast extract plus 10 mM thiosulfate	0.21	70
0.1% Yeast extract plus 10 mM thiosulfate	0.38	96
0.2% Yeast extract plus 10 mM thiosulfate	0.65	98
0.05% Glucose	0.22	
0.1% Glucose	0.46	
0.2% Glucose	0.71	
0.05% Glucose plus 10 mM thiosulfate	0.23	89
0.1% Glucose plus 10 mM thiosulfate	0.41	97
0.2% Glucose plus 10 mM thiosulfate	0.73	99

otrophs, the CO_2incorporation per cell was relatively unaffected by the presence of 0.01% yeast extract.

DISCUSSION

Our enrichment and isolation procedures employed conditions and media that have been previously successful in the detection and culti-

vation of terrestrial and marine sulfur-oxidizing bacteria; however, we cannot be certain that our approach resulted in the isolation of all physiological types of sulfur oxidizers present. For example, the various attached and filamentous bacteria that appear in scanning and transmission electron micrographs of microbial mats collected within the H_2S–O_2 regime of the vents resemble *Beggiatoa* and *Thiothrix*, but have resisted isolation (14). Such filamentous genera of sulfur oxidizers are notably difficult to culture (17), and only freshwater strains have so far been isolated in pure culture (20, 30).

The genus *Thiomicrospira* was originally described from estuarine environments (10, 19). The fact that we isolated this genus from the vents as the only obligate chemolithotroph may be related to its special physiological traits, i.e., its microaerophilic growth characteristics and tolerance for relatively high sulfide concentrations (18, 19; unpublished data). Of the 12 isolates, 8 were enriched in sulfide gradients, and all were indistinguishable from each other. The most successful inocula for enrichment of *Thiomicrospira* were pieces of mussel periostracum.

Acid-producing, heterotrophic, nonfermenting strains of small, motile or immotile, rod-shaped cells constituted a second group of sulfur-oxidizing bacteria isolated from a variety of thiosulfate-containing enrichments that were inoculated with either vent water samples, mussel periostracum or stomach contents, or surface scrapings of microbial mats. These organisms presumably oxidized thiosulfate to sulfate with a net increase in growth (cell numbers) and CO_2 incorporation characteristic of members of the genus *Thiobacillus*. Unlike typical thiobacilli (35), however, these isolates were unable to utilize CO_2 as the sole source of carbon. This physiological behavior can be described as chemolithoheterotrophic and has been found in *Thiobacillus perometabolis* (23). However, unlike in *T. perometabolis*, in our isolates high concentrations of organic compounds eliminated the thiosulfate enhancement of growth and CO_2 incorporation. Thus, only at a low concentration of dissolved organic compounds (such as might occur in the vent environment) does the chemolithotrophic activity of these strains appear to be a significant advantage to the organism.

The third group of isolates comprised the base-producing, heterotrophic, aerobic or facultatively anaerobic, rod-shaped bacteria that oxidized thiosulfate to polythionates without measurable enhancement of growth or CO_2 incorporation. Organisms of this type have been previously reported in other attempts to isolate thiobacilli from marine (34) and terrestrial (31) environments and, under special conditions, express a growth advantage by thiosulfate utilization (32, 33).

Many of our enrichments developed well in the absence of a combined nitrogen supplement. When 21 isolates of sulfur-oxidizing bacteria were tested for their capability to fix nitrogen, only one strain (NF-13; group V in Table 3) was found to be positive. A study of nitrogen-fixing isolates from the East Pacific Rise hydrothermal vent area is underway (J. Baross, personal communication).

None of the organisms we isolated from the vents has proved to be thermophilic. Because cells were found in high numbers in the emitted vent water (5, 13, 16), our working hypothesis is that autotrophic growth takes place within the vent system at or near-optimal growth temperatures (25°C in the case of *Thiomicrospira*) (unpublished data). Technically, it has not been possible to collect vent water that was undiluted by ambient seawater. An expedition planned for late 1981 will include improved sampling devices to allow the collection of water from deeper within the vents for use in further isolation attempts, including those for thermophilic bacteria, and for critical chemical determinations.

From recent studies (4a; H. Felbeck, Science, in press) it appears likely that chemosynthetic activity occurs in symbiotic associations between bacteria and certain vent invertebrates. Isolations from the possibly highly specialized microflora of the gill tissue of clams and the "trophosome" of vestimentiferan tube worms, both exhibiting ribulose bisphosphate carboxylase activity, will be part of future work with freshly collected material.

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