Thiosulfate Reduction, an Important Physiological Feature Shared by Members of the Order *Thermotogales*

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Several members of the order *Thermotogales* in the domain *Bacteria*, viz., *Thermotoga neapolitana*, *Thermotoga maritima*, *Thermosipho africanus*, *Fervidobacterium islandicum*, and *Thermotoga* strain SEBR 2665, an isolate from an oil well, reduced thiosulfate to sulfide. This reductive process enhanced cellular yields and growth rates of all the members but was more significant with the two hyperthermophiles *T. neapolitana* and *T. maritima*. This is the first report of such an occurrence in this group of thermophilic and hyperthermophilic anaerobic bacteria. The results suggest that thiosulfate reduction is important in the geochemical cycling of sulfur in anaerobic thermal environments such as the slightly acidic and neutral-pH volcanic hot springs and oil reservoirs.

Under oxic and anoxic conditions, chemical oxidation of sulfide results in the production of thiosulfate (6, 8, 9, 14, 15, 21), which acts as an intermediate during sulfur cycling in marine sediments (14). In the oxic parts of the sediments, thiosulfate can be oxidized by chemolithotrophs (e.g., *Thiobacillus* spp.) to sulfuric acid, while in the anoxic parts, thiosulfate disproportionation by sulfate-reducing bacteria occurs according to the following equation (14): $S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+$. Complete oxidation or disproportionation of thiosulfate decreases while reduction of thiosulfate increases with sediment depth (14), indicating that biological thiosulfate reduction is a highly significant process occurring in anoxic sediment environments.

Biological thiosulfate reduction is performed by mesophilic facultative anaerobes (e.g., members of the family *Enterobacteriaceae*) (4) and strict anaerobes (sulfate reducers) (4, 17) belonging to the domain *Bacteria*, but little information is available on thermophilic members of the domains *Archaea* (e.g., *Thermoproteus*, *Pyrodictium*, and *Pyrobaculum* spp.) and *Bacteria* (e.g., *Thermoanaerobacter* and *Thermoanaerobacterium* spp.) (16, 23). For the *Thermoanaerobacter* species, we have shown in our laboratory that the addition of thiosulfate to the growth medium improved the oxidation of proteinaceous compounds (e.g., peptides and amino acids [11]) and allowed H_2 consumption, thus alleviating its inhibitory effect on growth (10). In contrast to the situation for thiosulfate reduction, some attention has been paid to elemental sulfur reduction by thermophiles and hyperthermophiles in the domains *Archaea* and *Bacteria* (2, 16, 23). *Thermotoga neapolitana* and *Thermotoga maritima* are the only anaerobic hyperthermophiles belonging to the domain *Bacteria* known to reduce elemental sulfur to sulfide (12, 13).

In this paper, we report thiosulfate reduction by members of the order *Thermotogales*, including the hyperthermophiles *T.*

maritima and *T. neapolitana* and the thermophiles *Thermosipho africanus*, *Fervidobacterium islandicum*, and *Thermotoga* strain SEBR 2665. We present evidence that during thiosulfate reduction, there are considerable improvements in growth rates and biomass yields. We also comment on the important ecological role the process may play in mineralizing organic matter in thermophilic ecosystems.

Type cultures of members of the order *Thermotogales* were purchased from the German Culture Collection (DSM) and include *Thermosipho africanus* (DSM 5309), *F. islandicum* (DSM 5733), *T. maritima* (DSM 3109), *T. neapolitana* (DSM 4359), and a strain designated SEBR 2665, isolated from an oil field and identified as a *Thermotoga* species on the basis of the presence of typical "togas" and 16S rRNA sequence analysis (19). They were grown in 5 ml of basal medium that had been dispensed anaerobically into Hungate tubes. Basal medium contained the following: NH_4Cl, 1 g; K_2HPO_4, 0.3 g; KH_2PO_4, 0.3 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; cysteine-HCl, 0.5 g; KCl, 0.1 g; sodium acetate (anhydrous), 0.5 g; yeast extract, 5 g; bio-Trypcase, 5 g; trace mineral element (3), 10 ml; resazurin, 0.001 g; and distilled water, 1,000 ml. The pH was adjusted to 7.0. NaCl was added as follows for each strain: *F. islandicum*, 1 g/liter; *Thermosipho africanus*, 10 g/liter; and *T. maritima*, *T. neapolitana*, and strain SEBR 2665, 5 g/liter. After autoclaving of the tubes, (110°C, 20 min), 0.1 ml of 2% $Na_2S \cdot 9H_2O$, 0.1 ml of 10% Na_2HCO_3, and 0.1 ml of 1 M glucose were injected into each tube. When required, thiosulfate and sulfate were added as electron acceptors from sterile stock solutions to give the desired concentration. S^0 (sulfur sublimed, washed, and winnowed; Carlo Erba, Milan, Italy) was preweighted and added to the tubes prior to dispensing of the basal medium, and tubes were autoclaved as described above.

All cultures under investigation were able to ferment glucose and, in addition, were able to reduce sulfur but not sulfate as an electron acceptor during fermentation (Table 1), a characteristic that has already been reported in the literature (23). This trait of sulfur reduction is now extended to the nonvolcanic oil field isolate *Thermotoga* strain SEBR 2665 described

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TABLE 1. Thiosulfate reduction by members of the order *Thermotogales*

Organism	Dissolved sulfide produced (mM)				Maximal OD$_{580}$				Cellular productivity[a] (mg of cells [dry wt] · liter^{-1} · h^{-1})				Reduction[b]		
	Control	S^0 (2%)	Thiosulfate (20 mM)	Sulfate (20 mM)	Control	S^0 (2%)	Thiosulfate (20 mM)	Sulfate (20 mM)	Control	S^0 (2%)	Thiosulfate (20 mM)	Sulfate (20 mM)	S^0	Thio-sulfate	Sulfate
Fervidobacterium islandicum	1.5	19.3	24.6	2.2	0.9	1.1	1.8	0.9	14.6	19.5	38.1	12.0	+	+	−
Thermosipho africanus	1.4	23.7	24.6	1.3	0.9	1.0	1.3	0.9	23.8	27.0	36.0	23.0	+	+	−
Thermotoga maritima	1.5	19.7	14.4	1.3	0.3	0.5	1.2	0.3	5.2	12.1	21.1	5.2	+	+	−
Thermotoga neapolitana	1.5	27.7	13.6	1.7	0.4	0.6	1.2	0.4	6.5	14.7	21.3	7.7	+	+	−
Strain SEBR 2665	2.5	23.2	14.9	1.1	0.9	1.1	1.3	0.9	11.3	24.3	48.8	14.6	+	+	−

[a] OD$_{580}$, optical density at 580 nm. The following was calculated: 1 OD$_{580}$ unit = 0.5 g (dry weight) · liter^{-1}. Values were obtained after two subcultures in the same growth conditions.
[b] +, reduction; −, no reduction.

in this report (Table 1). All the strains, including the *Thermotoga* oil field isolate SEBR 2665, were also found to reduce thiosulfate, a feature that surprisingly has not been reported previously for this group of bacteria (Table 1).

During elemental sulfur reduction, the isolates produced significantly more H$_2$S than they did during thiosulfate reduction, with the exceptions of *F. islandicum* and *Thermosipho africanus*. The addition of thiosulfate and sulfur increased growth dramatically from that achieved without added electron acceptors. In thiosulfate-grown cultures, there was a 45% increase in growth for *Thermosipho africanus* and isolate SEBR 2665, a 100% increase for *F. islandicum*, and an average increase of 200% for both *T. maritima* and *T. neapolitana*, whereas in sulfur-grown cultures the increase ranged from 10% (*Thermosipho africanus*) to 65% (*T. maritima*) over growth in control cultures to which no electron acceptors had been added (Table 1). Together with increased densities, thiosulfate-grown cultures also had faster growth rates, as exemplified for *T. neapolitana* (Fig. 1), *T. maritima* (Fig. 2), and *F. islandicum* (Fig. 3). These growth improvements could partly result from a better utilization of peptides in the presence of thiosulfate. In addition, we observed that during glucose metabolism in the presence of thiosulfate, *T. africanus* and *F. islandicum* always produced more acetate than they did in its absence.

Members of the order *Thermotogales* possess numerous thermostable enzymes which could have industrial application (2, 18). However, biochemical studies (e.g., studies of enzyme production) for applied or fundamental purposes in general have been thwarted, as insufficient biomass is obtained. Therefore, expensive large-scale cultures, facilities for which are not always available to small laboratories, have to be grown. With results presented in this paper, it will now be possible to obtain adequate amounts of biomass for such studies by simply adding thiosulfate to the growth medium. The addition of thiosulfate, which is more soluble than traditionally used sulfur, is an
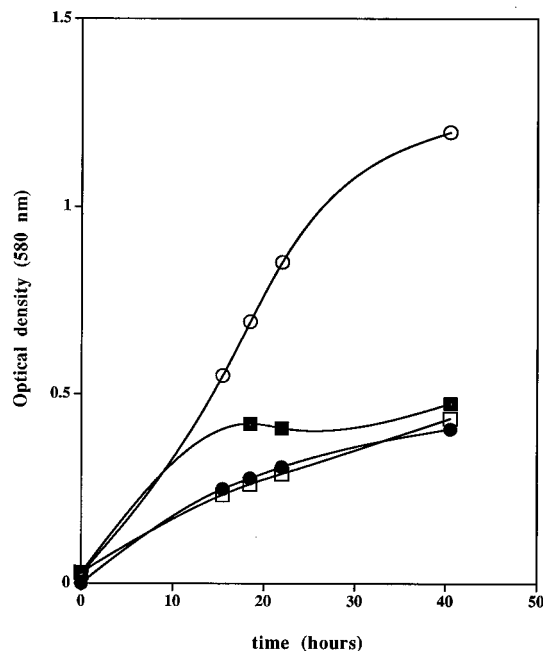

FIG. 1. Effect of electron acceptors on growth of *T. neapolitana*. ●, control; ■, S^0 (2%); ○, thiosulfate (20 mM); □, sulfate (20 mM).
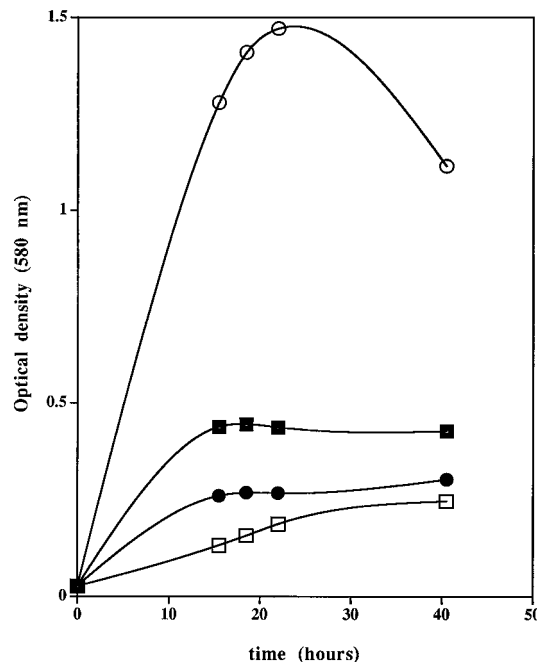

FIG. 2. Effect of electron acceptors on growth of *T. maritima*. ●, control; ■, S^0 (2%); ○, thiosulfate (20 mM); □, sulfate (20 mM).
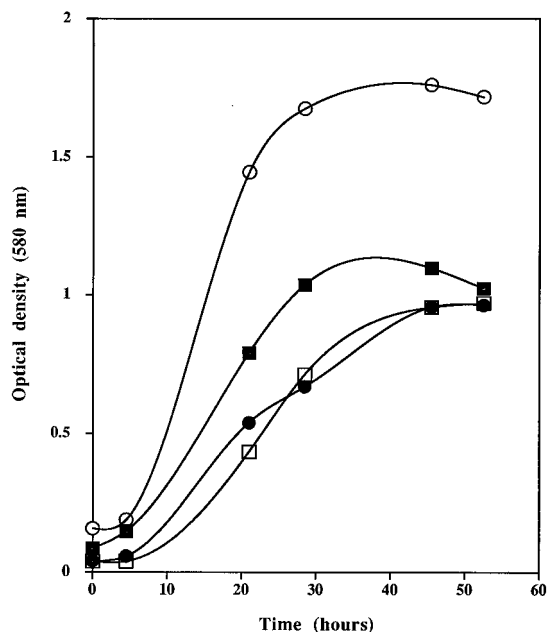
FIG. 3. Effect of electron acceptors on growth of *F. islandicum*. ●, control; ■, S^0 (2%); ○, thiosulfate (20 mM); □, sulfate (20 mM).

attractive alternative; furthermore, thiosulfate reduction could be less biocorrosive to industrial-scale stainless steel growth vessels, as reduced amounts of sulfide are produced with the same amount of substrate being oxidized.

The presence of members of the order *Thermotogales* (22, 24) and archaeal sulfate-reducing bacteria, viz., *Archaeoglobus* species, in oil fields (5, 24) and their ability to reduce thiosulfate to sulfide or to oxidize H_2 (1, 25) raise new questions about their involvement in biocorrosion of oil pipelines. Recently it has been hypothesized that the presence of thiosulfate could increase the risk of bacterial corrosion (7, 20). In addition, in the nonacidic continental and marine hot springs (23) and oil reservoirs, where conditions are anoxic and where thiosulfate oxidation is not possible or occurs only at slow rates, these thermophilic and hyperthermophilic thiosulfate reducers may play a major role in mineralizing organic matter (carbohydrates and peptides) in the presence of thiosulfate.

In our laboratory, we have recently isolated from oil field samples novel *Thermotoga* species which reduce thiosulfate and are inhibited by sulfur (22). From these results, we propose that for future microbiological ecological studies of oil fields and thermophilic environments, including terrestrial and marine systems, it is essential for thiosulfate to be incorporated as an electron acceptor in the medium, in addition to the conventional electron acceptors (sulfate and sulfur) currently being used, in order to study microbial diversity in such environments and for the isolation of novel microbes.

REFERENCES

1. **Adams, M. W. W.** 1990. The metabolism of hydrogen by extremely thermophilic, sulfur-dependent bacteria. FEMS Microbiol. Rev. **75:**219–238.
2. **Adams, M. W. W.** 1993. Enzymes and proteins from organisms that grow near and above 100°C. Annu. Rev. Microbiol. **47:**627–658.
3. **Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe.** 1979.

4. **Barrett, E. L., and M. A. Clark.** 1987. Tetrathionate reduction and production of hydrogen sulfide from thiosulfate. Microbiol. Rev. **51:**192–205.
5. **Beeder, J., R. K. Nilsen, J. T. Rosnes, T. Torsvik, and T. Lien.** 1994. *Archaeoglobus fulgidus* isolated from hot North Sea oil field waters. Appl. Environ. Microbiol. **60:**1227–1231.
6. **Cline, J. D., and F. A. Richards.** 1969. Oxygenation of hydrogen sulfide in seawater at constant salinity, temperature, and pH. Environ. Sci. Technol. **3:**838–843.
7. **Crolet, J. L., S. Daumas, and M. Magot.** 1993. pH regulation by sulfate-reducing bacteria, paper 303. *In* Corrosion 93. National Association of Corrosion Engineers, Houston, Tex.
8. **Crolet, J. L., M. Pourbaix, and A. Pourbaix.** 1991. The role of trace amounts of oxygen on the corrosivity of H_2S media, paper 22. *In* Corrosion 91. National Association of Corrosion Engineers, Houston, Tex.
9. **Cypionka, H., F. Widdel, and N. Pfennig.** 1985. Survival of sulfate-reducing bacteria after oxygen stress, and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiol. Lett. **31:**39–45.
10. **Fardeau, M. L., J. L. Cayol, M. Magot, and B. Ollivier.** 1993. H_2 oxidation in the presence of thiosulfate, by a *Thermoanaerobacter* strain isolated from an oil-producing well. FEMS Microbiol. Lett. **113:**327–332.
11. **Faudon, C., M. L. Fardeau, J. Heim, B. K. C. Patel, M. Magot, and B. Ollivier.** Peptide and amino acid oxidation in the presence of thiosulfate by members of the genus *Thermoanaerobacter*. Curr. Microbiol., in press.
12. **Huber, R., T. A. Langworthy, H. König, M. Thomm, C. R. Woese, U. W. Sleytr, and K. O. Stetter.** 1986. *Thermotoga maritima* sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90°C. Arch. Microbiol. **144:**324–333.
13. **Jannasch, H. W., R. Huber, S. Belkin, and K. O. Stetter.** 1988. *Thermotoga neapolitana* sp. nov. of the extremely thermophilic, eubacterial genus *Thermotoga*. Arch. Microbiol. **150:**103–104.
14. **Jørgensen, B. B.** 1990. A thiosulfate shunt in the sulfur cycle of marine sediments. Science **249:**152–154.
15. **Jørgensen, B. B.** 1994. Sulfate reduction and thiosulfate transformations in a cyanobacterial mat during a diel oxygen cycle. FEMS Microbiol. Ecol. **13:** 303–312.
16. **Lee, Y. E., M. K. Jain, C. Lee, S. E. Lowe, and J. G. Zeikus.** 1993. Taxonomic distinction of saccharolytic thermophilic anaerobes: description of *Thermoanaerobacterium xylanolyticum* gen. nov., sp. nov., and *Thermoanaerobacterium saccharolyticum* gen. nov., sp. nov.; reclassification of *Thermoanaerobium brockii*, *Clostridium thermosulfurogenes*, and *Clostridium thermohydrosulfuricum* E100-69 as *Thermoanaerobacter brockii* comb. nov., *Thermoanaerobacterium thermosulfurigenes* comb. nov., and *Thermoanaerobacter thermohydrosulfuricus* comb. nov., respectively; and transfer of *Clostridium thermohydrosulfuricum* 39E to *Thermoanaerobacter ethanolicus*. Int. J. Syst. Bacteriol. **43:**41–51.
17. **Le Faou, A., B. S. Rajagopal, L. Daniels, and G. Fauque.** 1990. Thiosulfate, polythionates and elemental sulfur assimilation and reduction in the bacterial world. FEMS Microbiol. Rev. **75:**351–382.
18. **Lowe, S. E., M. K. Jain, and J. G. Zeikus.** 1993. Biology, ecology, and biotechnological applications of anaerobic bacteria adapted to environmental stresses in temperature, pH, salinity, or substrates. Microbiol. Rev. **57:** 451–509.
19. **Magot, M.** Unpublished data.
20. **Magot, M., L. Carreau, J. L. Cayol, B. Ollivier, and J. L. Crolet.** Sulphide-producing, not sulphate-reducing anaerobic bacteria presumptively involved in bacterial corrosion. *In* C. A. C. Sequeira (ed.), Proceedings of the 3rd European Federation of Corrosion Workshop on Microbial Corrosion, in press. The Institute of Materials, London.
21. **Moses, C. O., D. K. Nordstrom, J. S. Herman, and A. L. Mills.** 1987. Aqueous pyrite oxidation by dissolved oxygen and by ferric iron. Geochim. Cosmochim. Acta **51:**1561–1571.
22. **Ravot, G., M. Magot, M. L. Fardeau, B. K. C. Patel, G. Prensier, A. Egan, J. L. Garcia, and B. Ollivier.** 1995. *Thermotoga elfii* sp. nov., a novel thermophilic bacterium from an African oil-producing well. Int. J. Syst. Bacteriol. **45:**308–314.
23. **Stetter, K. O., G. Fiala, G. Huber, R. Huber, and A. Segerer.** 1990. Hyperthermophilic microorganisms. FEMS Microbiol. Rev. **75:**117–124.
24. **Stetter, K. O., R. Huber, F. Blochl, M. Kurr, R. D. Eden, M. Fielder, H. Cash, and I. Vance.** 1993. Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. Nature (London) **365:**743–745.
25. **Zellner, G., E. Stackebrandt, H. Kneifel, P. Messner, U. B. Sleytr, E. C. De Macario, H. P. Zabel, K. O. Stetter, and J. Winter.** 1989. Isolation and characterization of a thermophilic, sulfate reducing archaebacterium, *Archaeoglobus fulgidus* strain Z. Syst. Appl. Microbiol. **11:**151–160.

Methanogens: reevaluation of a unique biological group. Microbiol. Rev. **43:**260–296.