CURRENT MICROBIOLOGY Vol. 29 (1994), pp. 269–272

Current Microbiology
An International Journal

Hydrogen Oxidation Abilities in the Presence of Thiosulfate as Electron Acceptor Within the Genus *Thermoanaerobacter*

Marie-Laure Fardeau,[1,2] Jean-Luc Cayol,[2] Michel Magot,[3] Bernard Ollivier[2]

[1]Laboratory of Bacterial Chemistry, National Center for Scientific Research, Marseille, France
[2]Laboratoire de Microbiologie ORSTOM, Université de Provence, 3 place Victor Hugo, 13331 Marseille Cedex 3, France
[3]Sanofi Research, Microbiology Unit, Labege, France

Abstract. *Thermoanaerobacter* (*T.*) *brockii*, *T. ethanolicus*, and *T. thermohydrosulfuricus* were tested for their capacities to oxidize H_2 in the presence of thiosulfate. *T. brockii* oxidized H_2 actively, while *T. ethanolicus* and *T. thermohydrosulfuricus* oxidized it poorly. At the end of the exponential growth, H_2 was oxidized by *T. brockii* in the presence of an energy source and thiosulfate. This oxidative process improved the growth of *T. brockii*. *Thermoanaerobacter* species could be divided into two groups with regard to their H_2 metabolism in the presence of thiosulfate. Thiosulfate reduction by species of the genus *Thermoanaerobacter* is of significance in mineralizing organic matter in thermophilic environments.

It has been stated recently that *Thermoanaerobacter finnii* and *Thermoanaerobacter* strain SEBR 5268, isolated from an oil-producing well [5], oxidized H_2 in the presence of thiosulfate when grown on yeast extract. *Thermoanaerobacter ethanolicus* [13] was described as the type strain of the genus *Thermoanaerobacter*, which was emended by including *T. brockii* and *T. thermohydrosulfuricus* [8].

Thermoanaerobacter differed from the genus *Thermoanaerobacterium* in reducing thiosulfate into sulfide, whereas *Thermoanaerobacterium* reduced it to elemental sulfur. Although the classification of the above species in the genus *Thermoanaerobacter* has been clearly evidenced, there was no report on their ability to reduce thiosulfate, particularly by *T. brockii* or *T. ethanolicus*. Furthermore, it was of interest to look for the capacity of these species to oxidize H_2 with regard to the results obtained by Fardeau *et al* . [5] on H_2 oxidation by *Thermoanaerobacter* strains.

We report on the ability of *T. brockii*, *T. ethanolicus*, and *T. thermohydrosulfuricus* to oxidize H_2 in the presence of thiosulfate.

Materials and Methods

Origin of strains. *Thermoanaerobacter thermohydrosulfuricus* No. 567, *T. brockii* No. 1457, and *T. ethanolicus* No. 2246 were obtained from DSM (Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany).

Correspondence to: B. Ollivier

Culture medium. All the experiments were performed at 60°C in the basal medium as described previously [5]. Yeast extract or biotrypcase was either added from separate autoclaved solutions or introduced directly in the medium. Glucose was added from filtered-sterilized stock solution and thiosulfate from autoclaved-sterilized solution. The anaerobic techniques of Hungate [7] were used throughout this work. After preparation of anaerobic media [5], liquid medium was distributed into Hungate tubes (5 ml) or serum bottles (50 ml). H_2 was brought in as a mixture of H_2/CO_2 (80/20, 2 bars).

Analytical techniques. Duplicate vessels were used during the course of this work. Optical density, hydrogen sulfide, and hydrogen were analyzed as previously described [5].

Results

H_2 oxidation in the presence of thiosulfate. In a first set of experiments, *Thermoanaerobacter brockii*, *T. thermohydrosulfuricus*, and *T. ethanolicus* were studied to evaluate their abilities to oxidize H_2. Significant growth was obtained with *T. brockii* when cultivated on yeast extract as the only energy source. No growth was observed with *T. thermohydrosulfuricus* and *T. ethanolicus* on yeast extract; therefore, these two strains were cultivated on a medium containing yeast extract supplemented with 5 mM of glucose as energy source.

Table 1. Effect of hydrogen addition on growth and sulfide production by *T. ethanolicus, T. thermohydrosulfuricus,* and *T. brockii* with and without thiosulfate

	Organisms					
	T. ethanolicus[b]		T. thermohydro-sulfuricus[b]		*T. brockii*	
Treatment[a]	OD[c]	H_2S[d]	OD[c]	H_2S[d]	OD[c]	H_2S[d]
M	0.244	0.77	0.446	0.73	0.080	2.31
M + H_2	0.174	0.24	0.302	0.46	0.022	3.59
M + TS	0.272	1.16	0.455	2.70	0.078	2.37
M + TS + H_2	0.148	2.5	0.423	4.40	0.180	28.15

[a] M, basal medium, containing 1 g/L yeast extract; H_2, hydrogen (H_2/CO_2: 80/20%, 2 bars); TS, thiosulfate (initial concentration was 20 mM).
[b] The culture medium contained 1g/L yeast extract and 5 mM glucose.
[c] Optical density was measured at 580nm after 3 days' incubation at 60°C.
[d] H_2S concentration (mM) was measured after 7 days' incubation at 60°C.

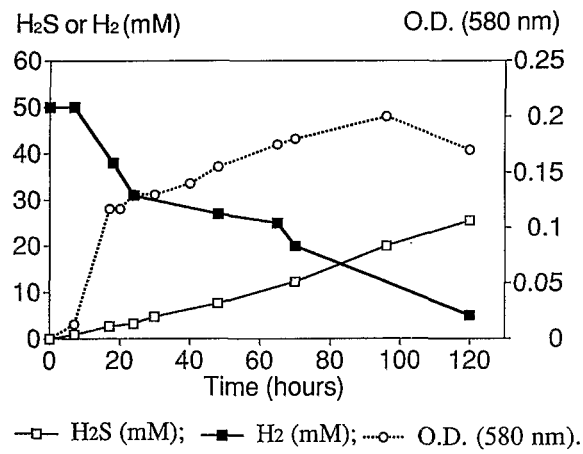

Fig. 1. H_2 oxidation by *T. brockii* in the presence of thiosulfate. (□) Sulfides; (■) hydrogen; (○) optical density.

T. brockii oxidized H_2 in the presence of thiosulfate-producing sulfide, while *T. ethanolicus* and *T. thermohydrosulfuricus* oxidized it slightly (Table 1). H_2 was oxidized by *T. brockii* after the exponential growth phase (Fig. 1). The addition of H_2 and thiosulfate improved the growth of *T. brockii* (Fig. 2), although H_2 was shown to be inhibitory for growth (Fig. 2).

Since sulfide production was low with *T. ethanolicus* in these experiments and because thiosulfate reduction by *T. ethanolicus* had never been physiologically evidenced, we decided to look for its capacity to produce sulfide by adding peptides and glucose as energy sources.

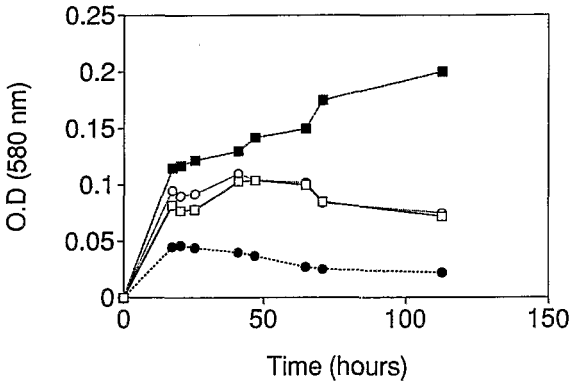

Fig. 2. Effect of H_2 addition on the growth of *Thermoanaerobacter brockii* in the presence or absence of thiosulfate. (○) Yeast extract; (□) yeast extract, thiosulfate; (●) yeast extract, hydrogen; (■) yeast extract, thiosulfate, hydrogen. Hydrogen was brought in as a mixture of H_2/CO_2 (80/20, 2 bars).

Table 2. Sulfide production with and without thiosulfate by *T. ethanolicus*

Growth conditions	OD[a] (580 nm)	H_2S[b] (mM)
yeast extract	0.012	1.01
yeast extract + biotrypcase	0.031	1.45
yeast extract + biotrypcase + thiosulfate	0.048	1.54
yeast extract + biotrypcase + glucose	0.669	1.81
yeast extract + biotrypcase + glucose + thiosulfate	0.446	6.20

T. ethanolicus was cultivated in the basal medium supplemented as follows: yeast extract and biotrypcase concentrations were 1g/L; thiosulfate and glucose concentrations were 20 mM.
[a] Optical density was measured after 2 days incubation at 60°C.
[b] H_2S production was measured after 5 days' incubation at 60°C.

T. ethanolicus produced small amounts of sulfide from biotrypcase in the absence of thiosulfate. In the presence of glucose, biotrypcase, and thiosulfate as electron acceptor, higher concentrations of sulfide were produced (Table 2).

Discussion

Recently, different thermophilic bacteria belonging to the genus *Clostridium* and *Thermoanaerobium* have been reclassified in the genus *Thermoanaerobacter*, which was characterized by its ability to reduce thiosulfate into sulfide [8]. H_2 oxidation in the presence of thiosulfate by *Thermoanaerobacter* strains was also recently demonstrated [5]. In this paper, we provide additional information about the ability to oxidize H_2 with other species of the genus *Thermoanaerobacter*. We demonstrate that *T. brockii* realized

this oxidative process, as described previously with *Thermoanaerobacter* 5268 [5]. First, *T. brockii* required an energy source to oxidize H_2. Furthermore, this activity was still important at the end of the exponential growth. In contrast, *T. ethanolicus* and *T. thermohydrosulfuricus* slightly oxidized H_2 compared with the two *Thermoanaerobacter* species listed above.

We report that *T. ethanolicus* produced small amounts of sulfide from biotrypcase fermentation. However, when glucose and biotrypcase were added to the medium, a higher sulfide production was obtained in the presence of thiosulfate. This indicates that *T. ethanolicus* produced sulfide from thiosulfate reduction or peptide fermentation, as described for *T. thermohydrosulfuricus* [6, 14].

When considering the metabolism of the five *Thermoanaerobacter* strains that we have studied (two in a previous report [5] and three in this study), it appears that species can be separated into two groups with regard to their H_2 metabolism. The first group, which contains *T. brockii* [15], *T. finnii* [12], and *Thermoanaerobacter* SEBR 5268 [5], actively oxidized H_2 in the presence of yeast extract as a potential energy source, while the second group, which contains *T. ethanolicus* [13] and *T. thermohydrosulfuricus* [6, 14], oxidized it poorly in the presence of yeast extract and glucose.

The results with regard to H_2 metabolism in the genus *Thermoanaerobacter* might be important from the physiological and taxonomical point of view. Thiosulfate reduction also evidences a probably important ecological role of this genus with regard to organic matter oxidation at high temperatures. Indeed, these microorganisms, which do not depend on an S-containing electron acceptor to ferment sugars, might have the opportunity to use thiosulfate to reach higher levels of sugar oxidation.

Barrett and Clark [2] suggested that thiosulfate reduction might also be significant for the survival of many bacteria. Although this was restricted to some aerobes or facultative anaerobes of the *Enterobacteriaceae* family, we can extend it to the anaerobic thermophilic thiosulfate reducers of the genus *Thermoanaerobacter*. These bacteria could use thiosulfate not only in mineralizing organic matter but also in maintaining favorable anoxic niches for growth by producing sulfide.

Thiosulfate can be obtained by chemical sulfide oxidation and thus might be present in anaerobic ecosystems when various concentrations of oxygen become available in these environments [3, 4]. The presence of thiosulfate could also result in a competition between other H_2 users such as sulfate reducers or methanogens, since it is known that in marine ecosystems sulfate reducers outcompete methanogens for H_2 in the presence of sulfate [10, 11].

Previous results did not evidence that H_2 oxidation was an energy-yielding reaction within *T. finnii* or *Thermoanaerobacter* SEBR 5268 [5]. In contrast, the current study shows that H_2 oxidation improves growth of *T. brockii* after the exponential growth phase. This indicates the occurrence of ATP synthesis during H_2 oxidation coupled with a concomitant reduction of thiosulfate. Since *T. brockii*, *T. finnii*, and SEBR 5268 are considerably inhibited by H_2 ([5], this study), H_2 oxidation probably plays a detoxifying role in the metabolism of these *Thermoanaerobacter* species. These metabolic features are similar to those described with some anaerobic hyperthermophiles isolated from deep-sea hydrothermal vents [1]. As suggested for the reduction of elemental sulfur with the above microorganisms [1], two mechanisms could be involved in the reduction of thiosulfate. One implies that the excess reductant might be coupled to thiosulfate reduction, while the second would amplify H^+ reduction. The involvement of thiosulfate reductase in this process and the recent discovery of a nonspecific elemental sulfur reductase acting as hydrogenase in *Pyrococcus furiosus* [9] raises the problem of the specificity of the thiosulfate reductase in the genus *Thermoanaerobacter* which might also act as a hydrogenase.

ACKNOWLEDGMENTS

We thank Dr. P.A. Roger for valuable discussions and critical comments on the manuscript.

Literature Cited

1. Adams MWW (1990) The metabolism of hydrogen by extremely thermophilic, sulfur-dependent bacteria. FEMS Microbiol Rev 75:219–238
2. Barrett EL, Clark MA (1987) Tetrathionate reduction and production of hydrogen sulfide from thiosulfate. Microbiol Rev 51:192–205
3. Crolet JL, Daumas S, Magot M (1993) pH regulation by sulfate-reducing bacteria. In: "Corrosion 93", paper N° 303, National Association of Corrosion Engineers, Houston, Texas USA
4. Cypionka H, Widdel F, Pfennig N (1985) Survival of sulfate-reducing bacteria after oxygen stress, and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiol Ecol 31:39–45
5. Fardeau ML, Cayol JL, Magot M, Ollivier B (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
6. Hollaus F, Sleytr U (1972) On the taxonomy and fine structure of some hyperthermophilic saccharolytic clostridia. Arch Mikrobiol 86:129–146
7. Hungate RE (1969) A roll tube method for cultivation of strict

anaerobes. In: Norris JR, Ribbons DW (eds) Methods in microbiology, vol 3B. London: Academic Press, pp 117–132

8. Lee YE, Jain MK, Lee C, Lowe SE, Zeikus JG (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 thermohydrosulfugenes* comb. nov., and *Thermoanaerobacter thermohydrosulfuricus* comb. nov., respectively; and transfer of *Clostridium thermohydrosulfuricum* 39E to *Thermoanaerobacter ethanolicus*. Int J Syst Bacteriol 43: 41–51
9. Ma K, Schicho RN, Kelly M, Adams MWW (1993) Hydrogenase of the hyperthermophile *Pyrococcus furiosus* is an elemental sulfur reductase or sulfhydrogenase: evidence for a sulfur-reducing hydrogenase ancestor. Proc Natl Acad Sci USA 90:5341–5344
10. Oremland RS, Polcin S (1982) Methanogenesis and sulfate reduction: competitive and non competitive substrates in estuarine sediments. Appl Environ Microbiol 44:1270–1276
11. Oremland RS, Marsh L, Polcin S (1982) Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediments. Nature 296:143–145
12. Schmid U, Giesel H, Schoberth SM, Sahm H (1986) *Thermoanaerobacter finnii* spec. nov., a new ethanologenic sporogenous bacterium. Syst Appl Microbiol 8:80–85
13. Wiegel J, Ljungdahl G (1981) *Thermoanaerobacter ethanolicus* gen. nov., sp. nov., a new, extreme thermophilic, anaerobic bacterium. Arch Microbiol 128:343–348
14. Wiegel J, Ljungdahl LS, Rawson JR (1979) Isolation from soil and properties of the extreme thermophile *Clostridium thermohydrosulfuricum*. J Bacteriol 139:800–810
15. Zeikus JG, Hegge PW, Anderson MA (1979) *Thermoanaerobium brockii* gen. nov. and sp. nov., a new chemoorganotrophic, caldoactive, anaerobic bacterium. Arch Microbiol 122:41–48