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Effect of thiosulphate as electron acceptor on glucose and xylose oxidation by *Thermoanaerobacter finnii* and a *Thermoanaerobacter* sp. isolated from oil field water

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SUMMARY

During glucose and xylose fermentation, *Thermoanaerobacter finnii* was observed to produce lactate, acetate, H_2 and CO_2, with ethanol being the major end product. *Thermoanaerobacter* strain SEBR 5268, an isolate from an oil field, also produced a similar range of end products from glucose and xylose fermentation, with the exception that both ethanol and lactate were the major products of sugar metabolism. Both these strains were able to reduce thiosulphate to sulphide in the presence of these two substrates, with acetate being the dominant metabolite in that case. In addition, a faster growth rate and increased cell yield were obtained in the presence of thiosulphate, than in its absence. The higher concentrations of acetate produced in the presence of thiosulphate rather than without any electron acceptor indicated that more ATP was generated from substrate-level phosphorylation. These results have implications for our understanding of the breakdown of carbohydrates present in organic matter found in the natural ecological niches of *Thermoanaerobacter* species (sulphide-, elemental sulphur- or sulphate-rich thermal hot springs and oil fields).

Key-words: Thiosulphate, Glucose, Xylose, *Thermoanaerobacter finnii*; Thiosulphate reduction, Anaerobiosis, Glucose and xylose oxidation, Thermal ecosystems, Oil fields.

INTRODUCTION

Several members of the genera *Thermoanaerobacter* and *Thermoanaerobacterium* of the "domain" *Bacteria* and members of the genera *Pyrodictium*, *Thermoproteus* and *Archeoglobus* of the "domain" *Archaea* are known to reduce thiosulphate, with concomitant production of sulphide (Stetter *et al.*, 1990; Lee *et al.*, 1993). Our recent studies have indicated that this process is also characteristic of all genera of the order *Thermotogales* (Ravot *et al.*, 1995), one of the deepest branches within the "domain" *Bacteria*.

It has recently been demonstrated that H_2 can also be oxidized in the presence of thiosulphate by members of the genus *Thermoanaerobacter*,

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which include *T. brockii*, *T. ethanolicus*, *T. thermohydrosulfuricus*, *T. finnii* and *Thermoanaerobacter* strain SEBR 5268, an isolate from an oil-producing well (Fardeau *et al.*, 1993, 1994). In contrast to *T. finnii*, in *T. brockii*, thiosulphate reduction coupled with H_2 oxidation participates in energy conservation (Fardeau *et al.*, 1994), as was observed for the hyperthermophilic sulphur-reducing *archaeon*, *Pyrococcus furiosus* (Schicho *et al.*, 1993). The addition of thiosulphate as an electron acceptor not only improves the oxidation of peptides, but also improves the oxidation of amino acids, thereby indicating that there is some form of dependency by *Thermoanaerobacter* species upon this electron acceptor during this process (Faudon *et al.*, 1995).

Most studies of *Thermoanaerobacter* species have focused on the large-scale growth on carbohydrates as fermentable sources for industrial applications, for example thermostable enzymes and alcohol production (Lowe *et al.*, 1993; Zamost *et al.*, 1991). No study of thiosulphate reduction and its implications for carbohydrate metabolism has been undertaken.

Despite this important and interesting trait of thiosulphate reduction by such a diverse group of microorganisms, more detailed attention has been paid to sulphur rather than to thiosulphate reduction (Adams, 1990; Kelly and Adams, 1994).

In this paper, we report on the effects of thiosulphate as an electron acceptor upon the oxidation of glucose and xylose by *T. finnii* and *Thermoanaerobacter* strain SEBR 5268, an oil field isolate. We also discuss the potential importance of this process in the oxidation of carbohydrates present in the organic fraction of the ecological habitats of these microbes, namely, thermal environments.

MATERIALS AND METHODS

Source of organisms

T. finnii (DSM no. 3389) was obtained from the Deutche Sammlung von Mikroorganismen. *Thermoanaerobacter* strain SEBR 5268 (DSM no. 9801) was isolated from an oil-producing well and has been deposited within the Sanofi-Recherche Collection of Microorganisms (Labège, France).

Media

Growth media were prepared using the technique of Hungate (1969). The organisms were cultivated under anaerobic conditions at 60°C. The basal medium contained the following constituents in distilled water (g/l): NH_4Cl, 1.0; K_2HPO_4, 0.3; KH_2PO_4, 0.3; NaCl, 2.0; $MgCl_2 \cdot 6H_2O$, 1.3; KCl, 0.2; $CaCl_2 \cdot 2H_2O$, 0.1; CH_3COONa, 0.5; yeast extract, 1.0; resazurin, 0.001; cystein. HCl, 0.5; trace mineral solution (Balch *et al.*, 1979), 10 ml; pH was adjusted to 7.0 with 10 M KOH. The medium was boiled under a stream of O_2-free N_2, cooled at room temperature and distributed in Hungate tubes (5 ml) or bottles (50 ml), which were outgassed with N_2-CO_2 (80/20 %). After autoclaving (110°C, 40 min), 0.2 ml of 2 % $Na_2S \cdot 9H_2O$ and 1 ml of 10 % $NaHCO_3$ (sterile, anaerobic conditions) were added for 20 ml of culture medium before inoculation. The basal medium was supplemented with glucose, xylose or thiosulphate at appropriate concentrations from sterile stock solutions. For CO_2 production measurements, an $NaHCO_3$-free medium was used with N_2 as the gas phase.

Analytical techniques

All experiments were performed in duplicate. Optical density (OD) was measured at 580 nm with a "UV-160A" spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Sulphide was quantified by the method of Cord-Ruwisch (1985). H_2 was measured as previously described (Fardeau *et al.*, 1993). CO_2 was analysed by gas chromatography at 70°C using a "Chrompack CP 9000" gas chromatograph (Chrompack International, Middelburg, The Netherlands), equipped with a catharometer and two columns filled with silicagel (60/80 mesh) and molecular sieve (60/80 mesh), respectively. Helium was used as a carrier gas. Sugars and fermentation products were quantified by high-performance liquid chromatography using an "Analprep 93" pump (Touzart et Matignon, Vitry sur Seine, France) equipped with a refractive index detector and an "ORH 801" column (Interaction Chemicals, Inc., Mountain View, CA.). Column temperature was 35°C. Eluant (H_2SO_4 0.005 N) was used at a flow rate of 0.6 ml/min.

RESULTS

Ethanol (32.7 mM) was the major end product of glucose fermentation by *T. finnii* at 60°C (table I and fig. 1A). In addition, acetate, lactate, H_2 (table I) and CO_2 (not shown) were also produced. Maximal concentrations of ethanol were

Table I. End products of glucose and xylose oxidation by *T. finnii* in the presence and in the absence of thiosulphate.

		Products of sugar metabolism					
Treatment	Sugar consumed (mM)	Acetate (mM)	Lactate (mM)	Ethanol (mM)	H_2S (mM)	H_2 (mM)	Acetate produced/ sugar consumed
Glu	22.5 ± 2.2	2.4 ± 0.2	17.8 ± 1.4	32.7 ± 0.5	0	2.7 ± 0.3	0.11
Glu + Thio	16.9 ± 0.2	18.2 ± 0.4	5.9 ± 1.0	14.6 ± 0.0	18 ± 1.2	0.2 ± 0.1	1.08
Xyl	21.2 ± 2.4	6.2 ± 1.2	7.3 ± 0.8	37.4 ± 0.0	0	3 ± 0.3	0.29
Xyl + Thio	16.7 ± 0.8	25.5 ± 1.7	7.5 ± 0.4	6.0 ± 0.0	25.9 ± 1.6	0.5 ± 0.2	1.53

T. finnii was cultivated at 60°C in the original medium described in "Materials and Methods". Thiosulphate was added at a concentration of 20 mM. Glu = glucose, Xyl = xylose, Thio = thiosulphate. The results were the mean of two replicates.

produced after 10 h of incubation, whereas lactate concentrations only peaked at 2 days of incubation at 60°C (fig. 1A). Similar end product profiles were obtained during xylose fermentation (table I and fig. 2A).

The addition of thiosulphate altered the concentrations of metabolites during glucose and xylose oxidation, indicating that thiosulphate modified the metabolic pathway of *T. finnii*. The changes observed were as follows: (i) thiosulphate was reduced to sulphide with concomitant reduction of H_2 to barely detectable levels (table I); (ii) the acetate concentration increased to such an extent that it became the major end product of glucose (table I and fig. 1B) and xylose (table I and fig. 2B) oxidation. A concomitant decrease in the production of ethanol was observed for glucose and xylose oxidation, whereas the lactate concentration decreased only when *T. finnii* was grown on glucose.

Modifications in the oxidoreductive pathway due to the presence of thiosulphate during glucose and xylose oxidation were observed to be beneficial for *T. finnii*: higher ODs were measured, indicating that (i) higher cell yield (table III) and (ii) a faster growth rate were achieved (table III).

Thermoanaerobacter strain SEBR 5268 produced an end product profile similar to that of *T. finnii*, but with the exception that ethanol and lactate were the major end products during glucose and xylose fermentation (table II). Cell yield increased and a faster growth rate was achieved (table III), with acetate becoming the major end product of carbohydrate metabolism in the presence of thiosulphate (table II), a phenomenon similar to that observed for *T. finnii*.

DISCUSSION

Thiosulphate reduction to sulphide is a common trait of sulphate-reducing bacteria (Barrett and Clark, 1987; Le Faou *et al.*, 1990). This feature is also observed amongst the carbohydrate-fermenting thermophilic anaerobic members of the genera *Thermoanaerobacter* and *Thermoanaerobacterium* (Lee *et al.*, 1993) and the order *Thermotogales* (Ravot *et al.*, 1995). We recently demonstrated that thiosulphate is used as an electron acceptor for oxidation of H_2, and it also improves the oxidation of peptides and amino acids by *Thermoanaerobacter* species (Fardeau *et al.*, 1993, 1994; Faudon *et al.*, 1995). We have extended these observations in this paper and have provided evidence that thiosulphate can be used as an electron acceptor, and dramatically modifies glucose and xylose metabolism in *T. finnii* and *Thermoanaerobacter* strain SEBR 5268, a strain isolated from a French oil field. Increased cell yield, faster growth rate and higher acetate production per mole of carbohydrate utilized in the presence of thiosulphate than in its absence suggest that this reductive process leads to a more favourable energetic reaction. We hypothesize that the use of thiosulphate as an electron

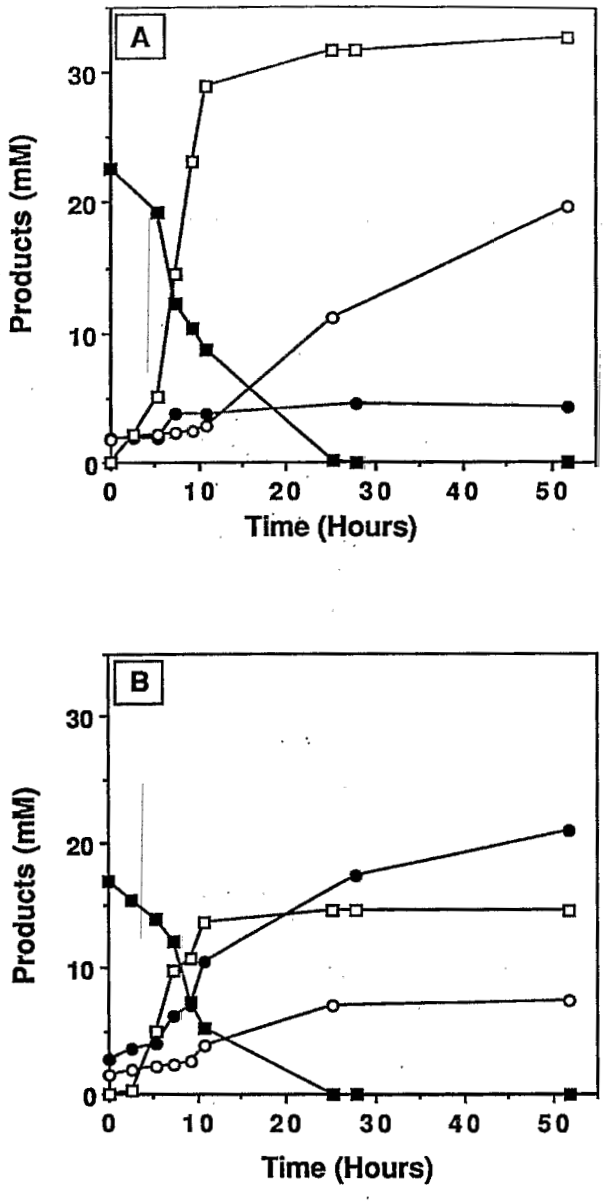

Fig. 1. End products of glucose oxidation in the absence (A) and in the presence of thiosulphate (B) by *T. finnii*: (■) glucose, (●) acetate, (□) ethanol and (○) lactate.

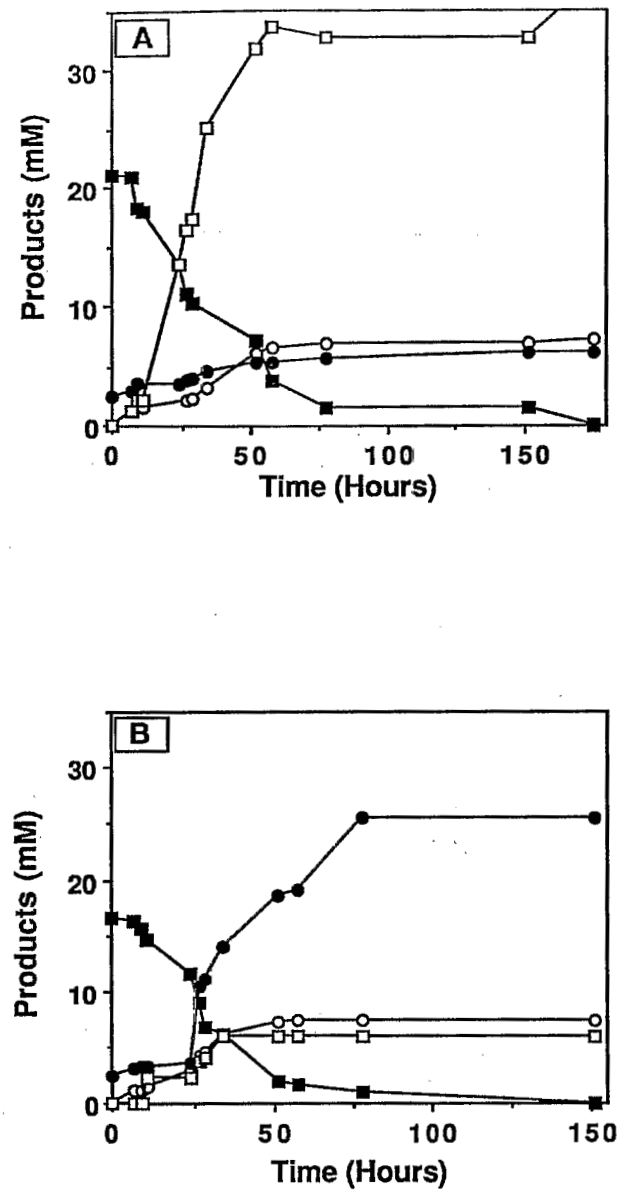

Fig. 2. End products of xylose oxidation in the absence (A) and in the presence of thiosulphate (B) by *T. finnii*: (■) xylose, (●) acetate, (□) ethanol and (○) lactate.

acceptor causes a shift in the flow of electrons, favouring H_2S production. This results in channelling of the electrons away from lactate and ethanol to acetate, thereby increasing its concentration. Such a shift in metabolism was previously reported, and was attributed to interspecies H_2 transfer (McInerney and Bryant, 1981; Mountfort *et al.*, 1982; Laube and Martin, 1981; Winter and Wolfe, 1979). Here, for the first time, we evidence this type of sugar metabolism in the presence of thiosulphate within members of the genus *Thermoanaerobacter*. In contrast to *T. finnii*, the archaeal *Thermoproteus tenax* used a limited amount of glucose in the presence of thiosulphate (or sulphur) and completely oxidized it to CO_2 and H_2S (Selig and Schönheit, 1994).

Table II. End products of glucose and xylose oxidation by strain SEBR 5268 in the presence and in the absence of thiosulphate.

		Products of sugar metabolism					
Treatment	Sugar consumed (mM)	Acetate (mM)	Lactate (mM)	Ethanol (mM)	H_2S (mM)	H_2 (mM)	Acetate produced/ sugar consumed
Glu	7.5 ± 0.4	ND	7.7 ± 2.5	7.2 ± 0.3	0	0.7 ± 0.1	0
Glu + Thio	7.4 ± 0.6	11.4 ± 1.9	5.4 ± 0.9	1.9 ± 0.2	2.7 ± 0.3	0.1 ± 0.1	1.54
Xyl	10.6 ± 0.5	1.1 ± 1.0	11.7 ± 2.1	9.6 ± 1.2	0	1.1 ± 0.2	0.10
Xyl + Thio	15.0 ± 1.5	18.0 ± 2.5	5.8 ± 1.6	4.3 ± 00	5.8 ± 0.4	0.3 ± 0.1	1.2

SEBR 5268 was cultivated at 60°C in original medium described in "Materials and Methods". Thiosulphate was added at a concentration of 20 mM. Glu = glucose, Xyl = xylose, Thio = thiosulphate. The results were the mean of two replicates. ND = not detected.

Table III. Effect of thiosulphate on growth of *Thermoanaerobacter* strains cultivated on glucose or xylose.

Strains	Treatments	Generation time (hours)	Maximal OD (580 nm)
T. finnii	Glucose	4	1.05
	Glucose + thiosulphate	2.6	1.40
	Xylose	5.9	0.77
	Xylose + thiosulphate	3.6	1.08
Thermoanaerobacter SEBR 5268	Glucose	6.9	0.85
	Glucose + thiosulphate	4.7	1.10
	Xylose	15.4	0.40
	Xylose + thiosulphate	5.5	1.25

Thermoanaerobacter strains were cultivated at 60°C. Glucose, xylose and thiosulphate were added at 20 mM to the original medium described in "Material and Methods".

The higher concentrations of acetate obtained from carbohydrate oxidation when thiosulphate is provided indicate that growth improvements result from more ATP generated from substrate-level phosphorylation. In the case of *T. finnii* and *Thermoanaerobacter* strain SEBR 5268, energy conservation via thiosulphate reduction as an alternative energy-yielding reaction can be ruled out, since we recently showed that no growth improvement resulted from concomitant oxidation of H_2 and reduction of thiosulphate to sulphide (Fardeau *et al.*, 1993). On the other hand, *T. brockii* might generate ATP during this oxido-reductive process (Fardeau *et al.*, 1994). This situation is analogous to that observed for the sulphur-reducing hyperthermophilic *Archaea*. Indeed some members of *Archaea* produced energy from elemental sulphur reduction, whereas others did not (Adams, 1990; Schicho *et al.*, 1993). Questions have been raised concerning the enzymatic and energetic systems involved in the reduction of sulphur by members of the *Archaea* (Kelly and Adams, 1994). On this basis, similar questions about the enzymatic and energetic systems involved in thiosulphate reduction can now be asked concerning members of the genus *Thermoanaerobacter*.

We recently demonstrated that thiosulphate reduction by members of the genus *Thermoanaerobacter* greatly improved the oxidation of

proteinaceous compounds, e.g. peptides and amino acids (Faudon *et al.*, 1995). Acetate, isobutyrate, isovalerate, small amounts of propionate and sulphide were detected as the end products of this metabolism. In the present paper, we have reported on modifications in the metabolism of carbohydrates in the presence of thiosulphate.

Thiosulphate is known to be formed chemically from sulphide in the presence of oxygen (Cypionka *et al.*, 1985; Jorgensen, 1990) or from elemental sulphur, anaerobically, at temperatures above 80°C (Pryor, 1962; Belkin *et al.*, 1985). Furthermore, it has recently been demonstrated that thiosulphate acts as a shunt in the sulphur cycle of marine sediments and that sulphate-reducing bacteria could reduce it or make it disproportionate (Jorgensen, 1990). In this respect, it can be hypothesized that, within thermophilic ecosystems (thermal hot springs and oil fields) which are rich in elemental sulphur, sulphide or sulphate and are the natural habitats for several *Thermoanaerobacter* species, efficient oxidation of carbohydrates and proteins can take place due to the presence of thiosulphate. Future studies on microbial ecology of thermal environments should take thiosulphate reduction into consideration so that a better understanding of mineralization processes can be obtained.

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Effet du thiosulfate comme accepteur d'électrons lors de l'oxydation du glucose et du xylose chez *Thermoanaerobacter finnii* et *Thermoanaerobacter* sp. isolé d'une eau de gisement pétrolier

Thermoanaerobacter finnii fermente le glucose et le xylose en lactate, acétate, H_2, CO_2 et éthanol qui est le produit majeur du métabolisme. Une autre bactérie du genre *Thermoanaerobacter* isolée d'une eau de gisement pétrolier produit des quantités aussi importantes d'éthanol que de lactate à partir de ces sucres. L'oxydation du glucose et du xylose par ces deux micro-organismes est complètement modifiée en présence de thiosulfate puisque, dans ces conditions, l'acétate devient le produit majeur du métabolisme des sucres. Cette modification du métabolisme amène à de meilleurs rendements cellulaires et à un meilleur taux de croissance bactérienne lorsque le thiosulfate est utilisé comme accepteur d'électrons. Une plus grande production d'acétate en présence de thiosulfate indique que les bactéries testées récupèrent plus d'énergie des phosphorylations liées au substrat. Ces modifications observées sur le métabolisme des sucres en présence de thiosulfate nous amènent à tenir compte plus précisément du rôle que peut jouer cet accepteur d'électrons dans l'oxydation de la matière organique dans les sources chaudes riches en sulfate et sulfure ou les eaux de gisements pétroliers.

Mots-clés: Thiosulfate, Glucose, Xylose, *Thermoanaerobacter finnii*; Réduction du thiosulfate, Anaérobiose, Oxydation du glucose et du xylose, Écosystèmes chauds, Puits de pétrole.

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