Extremophiles (2001) 5:45–51
Digital Object Identifier (DOI) 10.1007/s007920000172

ORIGINAL PAPER

Sigurlaug Skirnisdottir · Gudmundur O. Hreggvidsson
Olle Holst · Jakob K. Kristjansson

Isolation and characterization of a mixotrophic sulfur-oxidizing *Thermus scotoductus*

Received: May 8, 2000 / Accepted: November 14, 2000 / Published online: February 15, 2001

Abstract Thermophilic, faculatatively mixotrophic sulfur-oxidizing bacteria were isolated from a sulfide-rich, neutral hot spring in Iceland. The strain, IT-7254, used thiosulfate and elemental sulfur as electron donors, oxygen and nitrate as electron acceptors, and acetate and other organic compounds as carbon sources. After a few days of growth in the presence of thiosulfate, this strain formed sulfur globules. Comparison of intracellular enzymes and heme proteins of heterotrophically and mixotrophically grown cells showed some differences. The new isolate belonged to *Thermus scotoductus* because the small subunit (SSU) rRNA gene sequence analysis showed 98.6% sequence similarity and 84% DNA:DNA reassociation to *Thermus scotoductus* NMX2 A.1. It is also close to *Thermus antranikianii* HN3-7, with 98.3% and 79% SSU rRNA sequence similarity and DNA:DNA reassociation, respectively. It was also found that both *Thermus* NMX2 A.1 and T. *antranikianii* HN3-7 were able to oxidize thiosulfate but that the *T. scotoductus* type strain SE-1 was not. This is the first report of Thermus strains that are capable of mixotrophic growth with sulfur oxidation.

Key words Mixotrophic · Sulfur-oxidizing bacteria · Thermus · Hot spring

Communicated by J. Wiegel

S. Skirnisdottir · G.O. Hreggvidsson · J.K. Kristjansson (✉)
Prokaria Ltd., Gylfaflot 5, IS-112 Reykjavik, Iceland
Tel. +354-570-7900; Fax +354-570-7901
e-mail: jakob@prokaria.com

G.O. Hreggvidsson, J.K. Kristjansson
Institute of Biology, University of Iceland, Reykjavik, Iceland

S. Skirnisdottir, O. Holst
Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden

Introduction

Geothermal fields are commonly rich in inorganic reduced sulfur compounds such as hydrogen sulfide and elemental sulfur and, therefore, are particularly suited for thermophilic sulfur-oxidizing bacteria (Aragno 1992; Ehrlich 1996; Friedrich 1998). Relatively few thermophilic sulfur-oxidizing bacterial species have been isolated and described. Aragno (1992) divides sulfur-oxidizing thermophiles in the domain Bacteria into four main categories according to their metabolism. The first group contains hydrogen-oxidizing bacteria that also oxidize sulfur compounds. Among this group are obligate chemolithoautotrophic bacteria in the *Aquifex-Hydrogenobacter* group and the spore-forming facultative chemolithoautotroph *Bacillus schlegelii* (Alfredsson et al. 1986; Aragno 1992; Friedrich 1998; Friedrich and Mitrenga 1981; Schenk and Aragno 1979). Two *Thermothrix* species are known in the category of strictly thermophilic sulfur oxidizers (Caldwell et al. 1976; Odintsova et al. 1996). The third category includes moderately thermophilic, acidophilic *Thiobacillus*-like bacteria and the fourth category includes moderately thermophilic, strongly acidophilic sulfur- and iron-oxidizing bacteria (Aragno 1992).

Many active solfataric fields in southwest Iceland are located at low altitude and coincide with high groundwater level, giving rise to many freshwater hot springs of neutral pH and high sulfide concentrations. A characteristic feature of these hot springs is a thick white or gray bacterial mat in the runoff close to the source. For this study we selected a spring with a high sulfide concentration (12–20 $mg l^{-1}$), at 60°–75°C and with biomass several centimeters thick. A similar hot spring was described by Gorlenko and coworkers in the caldera of the Uzon volcano on Kamchatka, but no bacteria were isolated (Gorlenko et al. 1987). On the basis of the chemical composition of the hot spring, we assumed that the main primary producers would be hydrogen- and sulfur-oxidizing thermophiles. The purpose of this study was to isolate sulfur oxidizers and gain more information about the biomass that grows in this unique ecosystem.

On minimal medium gelrite plates containing thiosulfate, we isolated, as expected, bacteria belonging to the genera *Hydrogenobacter* and *Bacillus* but unexpectedly we also isolated bacteria of the genus *Thermus*.

Thermus strains have been isolated from different geothermal environments all around the globe, but until now all known bacteria belonging to the genus *Thermus* were thought be strict heterotrophs (Alfredsson and Kristjansson 1995; Williams and Sharp 1995). Although the bacteria belonging to the genus *Thermus* have been studied for decades, strains capable of sulfur oxidation have not been reported. Here, we describe the first facultatively mixotrophic sulfur-oxidizing *Thermus* strain, isolated from a sulfide-rich hot spring in Iceland.

Materials and methods

Study site and isolation

Filamentous bacterial biomass was collected from a sulfide-rich hot spring at 67°C and pH 6.7 in Grensdalur valley, 3 km due north from the town of Hveragerdi, Iceland. Other characteristics of the hot spring have been described elsewhere (Skirnisdottir et al. 2000). Three hours after sampling, samples from the bacterial mat were streaked on minimal medium gelrite plates containing 16 mM thiosulfate (16 mM thiosulfate medium) and incubated at 65°C for 2–4 days. Bright yellow colonies were picked and purified by repeated streaking onto the same medium.

Media and growth conditions

The 16 mM thiosulfate medium was prepared by adding 4 g l^{-1} thiosulfate ($Na_2S_2O_3 \times 5H_2O$) into minimal medium (Petursdottir and Kristjansson 1996). Four different nutrient media were used: medium R_2A (Reasoner and Geldreich 1985); medium 162 supplied with 2.5 g l^{-1} tryptone and 2.5 g l^{-1} yeast extract (Degryse et al. 1978); medium 160, which had the same composition as 162 except 1/10 phosphate buffer (Petursdottir and Kristjansson 1996), and medium 166, which contained 900 ml geothermal tap water, 100 ml mineral base (Petursdottir and Kristjansson 1996), 0.3 g K_2HPO_4 3H $_2O$, 1 g yeast extract, 1 g peptone, 1 g tryptone, 0.5 g glucose, 0.5 g starch, 0.6 g pyruvic acid, and 0.18 g Na_2CO_3. Utilization of single carbon sources was tested on agar plates or in liquid with minimal medium containing 0.2%–0.4% organic compounds as described previously (Kristjansson et al. 1994; Petursdottir and Kristjansson 1996). When gelrite was used as a carbon source in liquid cultures, it was solidified and autoclaved before addition. Solid media were prepared with 33 g l^{-1} gelrite (Phytagel, Sigma) or 28 g l^{-1} agar.

Growth tests and sulfate production

Heterotrophic growth was tested in media 160, 162, 166, and R_2A at 65°C. Growth was examined on the following single carbon sources: acetate, arginine, aspartate, casein, citrate, galactose, gelatine, glutamate, glucose, glycerol, lactose, maltose, phenylalanine, proline, pyruvate, serine, sorbitol, sucrose, starch, and valine. Chemolithoautotrophic growth was examined in liquid thiosulfate medium with no carbon source. Mixotrophic growth was tested in nutrient medium 166 supplied with 1, 2, 4, 8, or 16 mM thiosulfate and in thiosulfate medium (1, 2, 4, 8, or 16 mM) supplied with 0.15% acetate, arginine, aspartate, gelrite, glutamate, proline, or pyruvate. Chemolithoautotrophic growth on hydrogen was tested in H_2 medium under atmosphere of air plus 0.1 atm CO_2 and 0.6 atm H_2 (Alfredsson et al. 1986; Kristjansson et al. 1985). Sulfur globules were extracted from wet cells with pyridine as described by Nelson and Castenholz (1981).

The production of sulfate was tested in all liquid cultures containing thiosulfate. The production of sulfate from sulfur was tested in minimal medium with 0.15% acetate and sulfur. The concentration of sulfate was determined by using $BaCl_2$ as described by Tabatabai (1974). The changes in pH and growth density (absorbance at 620 nm) were followed in all cultures. Uninoculated controls were run in parallel to distinguish between chemical and biological oxidation of thiosulfate. *Thermus scotoductus* SE-1 (ATCC 51532; type strain), *Thermus antranikianii* HN3-7 (DSM 12462; type strain), and *T. scotoductus* NMX2 A.1 (kindly provided by Hugh Morgan, University of Waikato, Hamilton, New Zealand) were used as reference strains for all growth tests.

Phenotypic characterization

All tests were carried out on cultures grown in nutrient medium 166 if not stated otherwise. The range and optimum growth temperature were examined at 48°, 50°, 55°, 60°, 65°, 68°, 72°, and 75°C for 4 days. Growth was examined at pH 4.5, 5.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, and 9.5 with appropriate buffers for 4 days at 65°C (Breznak and Costilow 1994). Salt tolerance was tested with 0.5% and 1% NaCl. Presence of catalase activity was determined by the formation of bubbles with 3% (v/v) hydrogen peroxide solution, and oxidase activity was determined by the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Smibert and Krieg 1994). Nitrate reduction was tested with 4-day-old cultures (Smibert and Krieg 1994). The presence of spores was examined after 5 days of growth according to the Schaeffer–Fulton method (Murray et al. 1994). Bacteria grown on 16 mM thiosulfate gelrite medium and nutrient medium 166 supplied with 16 mM thiosulfate were analyzed by phase-contrast microscopy and transmission electron microscopy. For transmission electron microscopy, cells were processed as described previously (Kristjansson et al. 1994).

Analyses of intracellular enzymes and cytochromes

Intracellular enzymes were analyzed by multilocus enzyme electrophoresis (MEE). Bacteria were cultivated in nutrient medium 166 and on 16 mM thiosulfate gelrite plates. *T.*

scotoductus SE-1 was only grown in medium 166 as it could not grow on thiosulfate medium. Cells were harvested from liquid cultures by centrifugation (15,000 *g* for 15 min) or scraped from plates, then suspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), giving about 1 g in 5 ml, and then lysed in a French press at 700 psi. The crude extract was centrifuged at 30,000 *g* for 30 min and the supernatant collected and kept at −80°C until use.

Before use, the samples were centrifuged again and the clear supernatant collected. The samples were run on 7.5% (w/v) polyacrylamide gels; the gels were then incubated in eight different enzyme solutions (Manchenko 1994; Selander et al. 1986). The following enzymes were tested: aspartate aminotransferase (AAT), fructose bisphosphatase (FBP), glucose-6-phosphate isomerase (GPI), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hexokinase (HK), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), and phosphoglucomutase (PGM). Superoxide dismutase (SOD) was visual on all dehydrogenase-staining gels, thus giving the ninth marker. Membrane-bound and cytoplasmic cytochromes were determined in the pellet and the clear supernatant. The samples were run on 12.5% (w/v) polyacrylamide gels with 0.1% SDS as previously described (Petursdottir and Kristjansson 1997). LMW markers from Amersham Pharmacia Biotech (Uppsala, Sweden) were used for size determination.

DNA:DNA reassociation studies and DNA base composition

DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (1977). DNA:DNA reassociation studies were carried out between IT-7254 and to each of the three following strains: *T. scotoductus* SE-1, *T. antranikianii* HN3-7 and *T. scotoductus* NMX2 A.1 as described by De Ley et al. (1970), with modifications described by Huss et al. (1983) and Escara and Hutton (1980). Analysis of the G+C content was performed at DSMZ using standard methods (Cashion et al. 1977; Mesbah et al. 1989).

Phylogenetic analysis

DNA was isolated with a Dynabeads DNA Direct Kit (Dynal Biotech, Oslo, Norway) according to the manufacturer. By using DyNAzyme polymerase (Finnzymes, Espoo, Finland) as described by the manufacturer, PCR amplification of the small subunit (SSU) rRNA gene was performed. The primer set consisted of F9 and R1544 (Skirnisdottir et al. 2000). The reactions were as follows: 25 cycles at 95°C for 50 s, 52°C for 50 s, and 72°C for 3 min. Before sequencing, the PCR products were purified with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) as described by the manufacturer. The sequence was determined by using the dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA) according to the manufacturer. The following sequence primers were used: F9, F338, F515, F814, F1392, R357, R805, R1195, and R1544 (Pitulle et al. 1994; Skirnisdottir et al. 2000). After a BLAST search, the sequence was aligned with other sequences within the *Thermus* group obtained from the Ribosomal Database Project (Maidak et al. 1999) and by using the ARB database alignment from the Department of Microbiology, the Technical University in Munich, Germany (S. Strunk and W. Ludwig, http://www.mikro.biologie.tu-muenchen.de/pub/ARB/). Homologous nucleotide positions, based on the filter of the ARB database, were included in the alignment and used for the comparative analysis. Evolutionary distances were computed from pairwise similarities using the correction of Jukes and Cantor (Jukes and Cantor 1969). Distance trees were constructed by the neighbor-joining algorithm. The nucleotide sequence of strain IT-7254 has been deposited in the GenBank database under the accession number AF257219.

Results

Growth and sulfate production

After 2–4 days incubation on 16 mM thiosulfate gelrite medium at 65°C, bright yellow colonies that were 0.5–1 mm in diameter appeared. They were purified on the same medium, and all further experiments were done on strain IT-7254. Streaking on control plates with the same medium without thiosulfate did not result in growth. The bacteria grew well on thiosulfate medium gelrite plates but not on thiosulfate agar plates. Of the heterotrophic media tested (on agar plates and in liquid), the bacteria grew best on nutrient medium 166. They grew poorly on nutrient media 160 and R_2A and did not grow on nutrient medium 162. On nutrient agar medium 166, the bacteria formed 0.5–1 mm light yellow colonies after 2–3 days at 65°C. Of the 20 different compounds tested as single carbon sources, strain IT-7254 was able to utilize arginine, aspartate, glutamate, proline, and pyruvate. Strain IT-7254 and the reference strains were not able to grow on hydrogen.

Growth of IT-7254 on thiosulfate resulted in formation of sulfate in all types of media tested but varied depending on the medium used (3–6 mM sulfate was produced from 16 mM thiosulfate after 2–10 days). Thiosulfate enhanced growth in all liquid media tested, and it seemed that the bacteria preferred mixotrophic growth, as they grew better in nutrient medium 166 and in minimal medium with single carbon sources (acetate, arginine, aspartate, glutamate, proline, or pyruvate) supplied with thiosulfate than without it. The strain was able to oxidize sulfur to sulfate in the presence of acetate (2.3 mM sulfate produced after 4 days) but less efficiently than with thiosulfate (5.6 mM sulfate from 16 mM thiosulfate after 2 days). In liquid thiosulfate medium with no carbon source (chemolithoautotrophic conditions), the bacteria grew extremely poorly but precipitated sulfur after approximately 10 days. Therefore, it was not possible to use direct spectrophotometry to measure the increase in biomass formation. Although some increase in

Table 1. Results of main growth tests in minimal medium of strain IT-7254 in liquid, on agar, and on gelrite plates

Component added	Gelrite plates	Agar plates	Liquid
Thiosulfate	+++	−	(−)
Gelrite	−	nd	−
Thiosulfate and gelrite	+++	nd	++
Acetate	−	−	−
Thiosulfate and acetate	nd	+++	+++
Sulfur	nd	nd	−
Sulfur and acetate	nd	nd	++

nd, not done

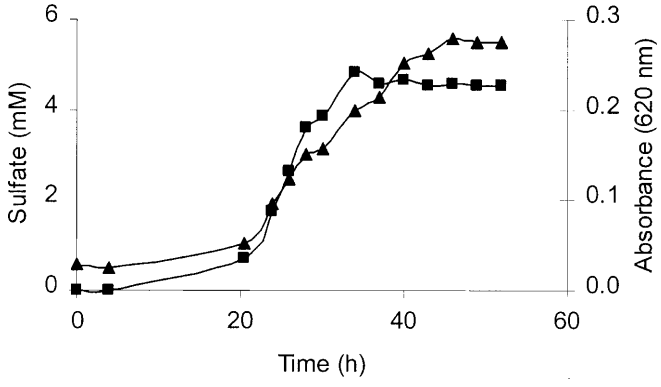

Fig. 1. Production of sulfate (*triangles*) and changes in optical density (*squares*) by strain IT-7254 growing mixotrophically in 16 mM thiosulfate medium supplied with 0.15% acetate at 65°C

cell counts and sulfate production was seen in these cultures, chemolithoautotrophic growth could not be unequivocally demonstrated. The results of the main growth tests for strain IT-7254 are shown in Table 1.

The bacteria grew best and produced the most sulfate in thiosulfate medium supplied with acetate (Fig. 1) and did not grow on acetate without thiosulfate. When the initial concentration of thiosulfate was 1, 2, 4, 8, and 16 mM in this medium, the sulfate produced after 46 h was 1.9, 3.1, 4.9, 5.4, and 5.6 mM, respectively; this corresponded to a conversion of 95%, 78%, 61%, 34%, and 18%, respectively, of the total available sulfur to sulfate. Thus, at limiting concentrations of thiosulfate ($S_2O_3^{2-}$), both S atoms were oxidized to sulfate. Sulfur precipitations were usually seen in the bottom of liquid culture bottles with the highest thiosulfate concentration. The initial pH in the two highest thiosulfate concentrations with acetate was about 7.6, and this value decreased to about 6.1 during growth. In cultures with 1, 2, and 4 mM thiosulfate, the pH slightly increased. Thiosulfate medium with other single carbon sources and nutrient medium 166 plus thiosulfate (1, 2, 4, 8, and 16 mM) gave similar results as with acetate, except that longer growth periods were needed (6–10 days).

Sulfate production in uninoculated controls as well as in *T. scotoductus* SE-1 cultures was analyzed in parallel and was found to be negligible. The reference strains, *T. antranikianii* HN3-7 and *T. scotoductus* NMX2 A.1, were able to oxidize thiosulfate to sulfate, giving 2.7 and 6.7 mM sulfate, respectively, after 46 h growth from 16 mM thiosulfate. Although the two strains were able to oxidize thiosulfate, they grew better on acetate alone than in the same minimal medium supplied with thiosulfate. Therefore, mixotrophic growth could not be unequivocally demonstrated for these strains.

In nutrient medium 166, the bacteria grew as long filaments, 50–200 × 0.7 µm. In thiosulfate medium with single carbon sources, the cells were smaller and single, 3–4 × 0.7 µm. After 7–9 days incubation in nutrient medium 166 with thiosulfate, part of the cells formed small sulfur globules (1 µm) along the long axis of the filaments, and the size of the globules increased for the next 3–4 days, when they disappeared. The average size of the globules was about 15 µm; however, some globules were as much as 150 µm long (Fig. 2a,b). Less than 5% of the filaments contained those globules. Most had 3 to 6 globules each, although the longest filaments had 10 to 15. Similar but much smaller sulfur globules appeared in thiosulfate medium (Fig. 2c). After growth on 16 mM thiosulfate gelrite medium, a dense peptidoglycan layer as well as some invaginated pockets of the cytoplasmic membrane were seen in transmission electron photomicrographs (Fig. 2d). That these globules were indeed sulfur was supported by a number of observations: (i) they were only produced in medium supplemented with thiosulfate; (ii) the globules were highly refractile when viewed in phase-contrast microscopy; and (iii) they could be extracted with pyridine. *T. scotoductus* SE-1 did not form these sulfur globules but the other two reference strains did.

Phenotypic characterization

The bacteria grew well between 60° and 68°C, and slowly at 50°–55°C and at 72°C. They did not grow below 50°C or above 75°C. The optimum growth temperature was 65°C. The growth rate was similar at pH 6.5 to 8.5, and slightly lower at pH 9.0, but no growth occurred at pH 5.5 or 9.5. The bacteria grew in 0.5% NaCl but not at higher salt concentrations. The bacteria were gram negative, nonspore forming, and positive for oxidase, catalase, and nitrate reduction.

Intracellular enzymes and cytochromes

Comparison of intracellular enzymes in strain IT-7254 and *T. scotoductus* SE-1 is shown in Table 2. Strain SE-1 expressed all the enzymes tested but the expression of the enzymes of strain IT-7254 depended on the medium. The cytochrome staining of the membrane fraction revealed three cytochromes of the same sizes after growth on both thiosulfate medium and nutrient medium 166, but two additional cytochromes were expressed after growth on thiosulfate medium. However, different cytoplasmic cytochromes were expressed after growth on thiosulfate medium and nutrient medium 166.

Phylogeny and DNA:DNA relatedness

The SSU rRNA gene sequence was determined. Phylogenetic analysis showed that strain IT-7254 clustered most

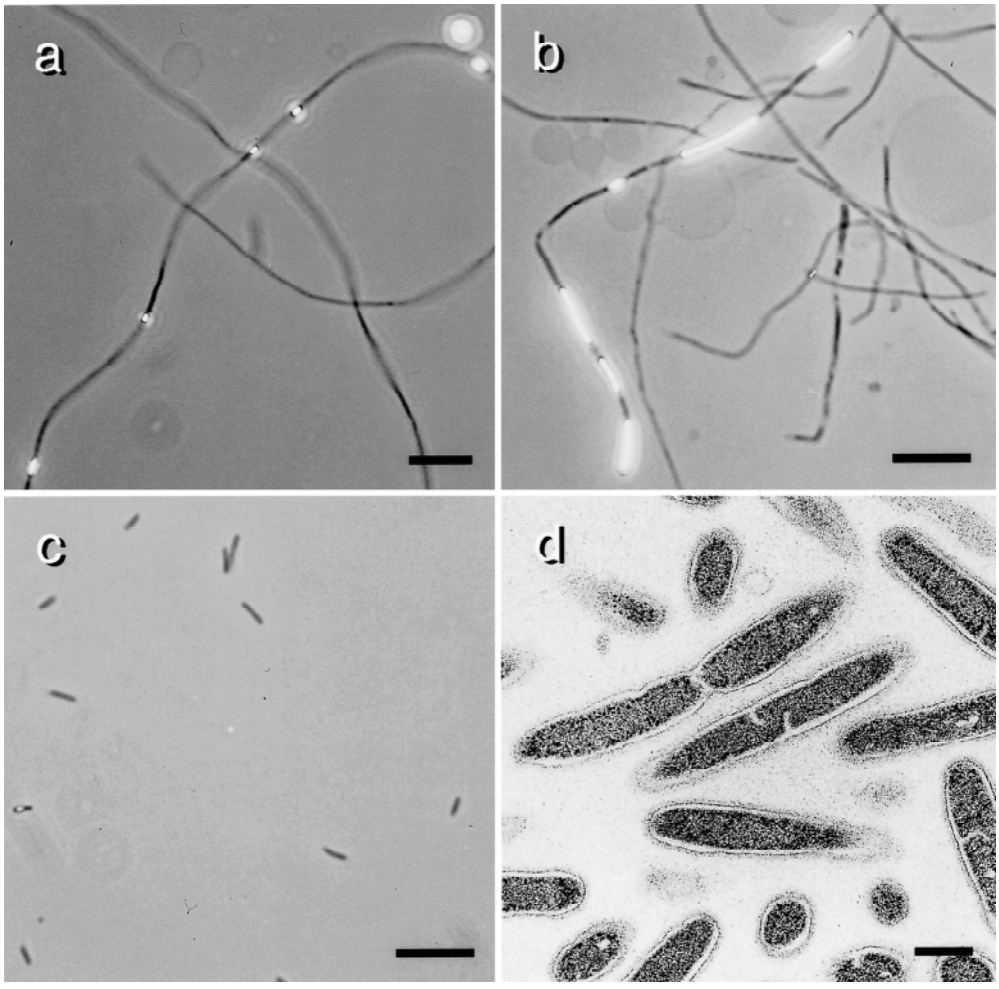

Fig. 2a–d. Phase-contrast (**a–c**) and electron (**d**) photomicrographs of IT-7254. **a, b** Bacterial filaments containing sulfur globules after growth in nutrient medium 166 supplied with 16 mM thiosulfate. **a** Initial formation of globules. *Bar* 10 µm. **b** Average size of fully formed sulfur globules. *Bar* 10 µm. **c** Single cells with sulfur globules after growth on 16 mM thiosulfate medium. *Bar* 10 µm. **d** Thin sections of cells after growth on 16 mM thiosulfate medium showing invaginal pockets. *Bar* 0.5 µm

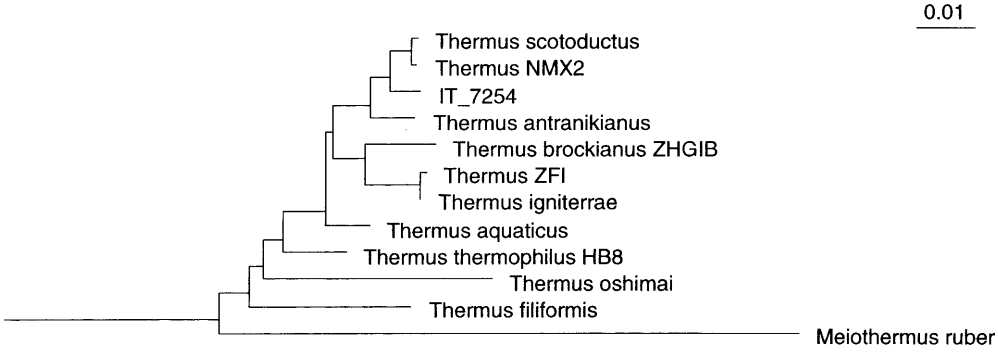

Fig. 3. Phylogenetic position of strain IT-7254 among members of *Thermus* based on SSU rRNA sequencing. *Bar* represents 1% sequence divergence

closely with members of the genus *Thermus.* It showed 98.6% sequence similarity with *T. scotoductus* NMX2 A.1, and 98.3% with both *T. scotoductus* SE-1 and *T. antranikianii* HN3-7. A distance tree comprising the new isolate in the context of currently recognized species of the genus *Thermus* is shown in Fig. 3. The DNA:DNA reassociation of *Thermus* IT-7254 was 84% with *T. scotoductus* NMX2 A.1, but it was 77% and 79% with *T. scotoductus* SE-1 and *T. antranikianii* HN3-7, respectively. The DNA base composition of strain IT-7254 was 65.9 mol% G+C.

Discussion

Until now, it has been believed that all bacteria belonging to the genus *Thermus* were strict heterothrophs (Williams and Sharp 1995). Therefore, this is the first report of sulfur-oxidizing representatives in this genus. The SSU rRNA sequencing and DNA:DNA reassociation studies placed the new facultative mixotrophic sulfur-oxidizing thermophile IT-7254 in the species *T. scotoductus*, but it is also closely related to *T. antranikianii* (Fig. 3). This

Table 2. Expression of intracellular enzymes by *Thermus scotoductus* SE-1 and strain IT-7254 grown in nutrient medium 166 and in thiosulfate medium

Enzyme	T. scotoductus SE-1 166 medium	IT-7254 166 medium	IT-7254 Thiosulfate medium
Aspartate aminotransferase	1	1	1
Hexokinase	1	1	1
Superoxide dismutase	1	1	1
Glucose-6-phosphate isomerase	1	2	2
Malate dehydrogenase	1	1, 2	1, 2
Fructose bisphosphatase	1, 2	1, 2	1, 3
Isocitrate dehydrogenase	1	1	0[a]
Phosphoglucomutase	1	0[a]	0[a]
Glyceraldehyde-3-phosphate dehydrogenase	1	0[a]	0[a]

Data are number of allozymes detected
[a]No band visualizedm

placement is supported by the MEE analysis (Table 2).

Chemolithoautotrophic growth could not be unequivocally demonstrated for IT-7254 as the bacteria grew extremely poorly under these conditions. However, by adding a carbon source (gelrite, acetate, arginine, aspartate, glutamate, proline, or pyruvate) into the corresponding liquid medium, the growth was significantly enhanced. Thus, it appears that the strain preferred mixotrophic growth because the bacteria grew better in medium containing both thiosulfate (or sulfur) and a carbon source than in media containing only one of these components. The fastest growth and greatest sulfate formation was in medium with thiosulfate and acetate. No growth occurred in minimal medium with only acetate, but growth was significantly enhanced with acetate in the presence of thiosulfate or sulfur. Therefore, this strain seems to be using acetate as a carbon source but not as an energy source. The same growth pattern was seen with gelrite, indicating that the strain was able to assimilate organic compounds that were present in the gelrite.

Thermus scotoductus NMX2 A.1 and *T. antranikianii* HN3-7 grew better on acetate alone than on acetate and thiosulfate together. Because they also oxidized thiosulfate to sulfate in the presence of carbon sources, they were probably also growing mixotrophically. We analyzed five other *T. scotoductus* strains from our strain collection (detected by SSU rRNA sequencing and MEE analysis) for this property. Two of them were positive, indicating that this property may be widespread among *Thermus* spp. (unpublished observations). Strain IT-7254, *T. scotoductus* NMX2 A.1, and *T. antranikianii* HN3-7 formed sulfur globules in the presence of thiosulfate. As in the case with sulfur-oxidizing *Chloroflexus*, it appeared that they were loosely attached because similar sulfur globules floated in the medium, indicating that they were external. The floating globules could also have come from dead cells (Brock 1978).

The analysis of intracellular enzymes showed that the metabolism of IT-7254 differed from that of *T. scotoductus* SE-1 (Table 2). IT-7254 had no or very little expression of PGM and GAPDH. Moreover, IT-7254 seemed to have a different metabolism when growing heterotrophically and mixotrophically. The bacterium did not express IDH, one of the key enzymes in the TCA cycle, when grown mixotrophically. It can use acetate and other organic substrates as carbon sources during mixotrophy. Acetate assimilation has been observed in *Chloroflexus*, but these bacteria can form acetyl CoA from acetate directly by acetyl CoA synthetase (Brock 1978; Hole and Grace 1987; Mathews and van Holde 1990; Sirevaag 1992). Strain IT-7254 expressed two extra cytochromes when growing on thiosulfate medium, indicating that different cytochrome systems were used during sulfur oxidation.

Recently, a *T. scotoductus* strain was isolated from a depth of 3.2 km in a South African gold mine. It was able to use Fe^{3+}, S^0, NO_3^-, and O_2 as terminal electron acceptors for heterotrophic growth (Kieft et al. 1999). However, these authors did not test their isolate for autotrophic or mixotrophic growth. In addition to our discovery of a faculatively mixotrophic sulfur-oxidizing *Thermus*, these results indicate that the diversity and metabolism of *Thermus* spp., and especially of *T. scotoductus*, are greater than were previously believed.

Acknowledgments We thank Jóhann Arnfinnsson, University of Iceland for the electron microscopy and Hugh Morgan, University of Waikato, Hamilton, New Zealand, for providing Thermus strain NMX2 A.1. This work was supported by The European Union, grant no. Bio4-CT96–0488.

References

Alfredsson GA, Kristjansson JK (1995) Ecology, distribution and isolation of *Thermus.* In: Sharp R, Williams R (eds) *Thermus* species. Plenum, New York, pp 43–66

Alfredsson GA, Ingason A, Kristjansson JK (1986) Growth of thermophilic obligately autotrophic hydrogen-oxidizing bacteria on thiosulfate. Lett Appl Microbiol 2:21–23

Aragno M (1992) Aerobic, chemolithoautotrophic, thermophilic bacteria. In: Kristjansson JK (ed) Thermophilic bacteria. CRC Press, Boca Raton, pp 77–103

Breznak JA, Costilow RN (1994) Physicochemical factors in growth. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 137–154

Brock TD (1978) Thermophilic microorganisms and life at high temperatures. Springer, New York

Caldwell DE, Caldwell SJ, Laycock JP (1976) *Thermothrix thioparus* gen. et sp. nov.: a facultatively anaerobic facultative chemolithotroph living at neutral pH and high temperature. Can J Microbiol 22:1509–1517

Cashion P, Holder-Franklin MA, McCully J, Franklin M (1977) A rapid method for the base ratio determination of bacterial DNA. Anal Biochem 81:461–466

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Degryse E, Glansdorff N, Pierard A (1978) A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. Arch Microbiol 117:189–196

Ehrlich HL (1996) Geomicrobiology. Dekker, New York

Escara JF, Hutton JR (1980) Thermal stability and renaturation of DNA in dimethylsulphoxide solutions: acceleration of the renaturation rate. Biopolymers 19:1315–1327

Friedrich CG (1998) Physiology and genetics of sulfur-oxidizing bacteria. Adv Microb Physiol 39:235–289

Friedrich CG, Mitrenga G (1981) Oxidation of thiosulfate by *Paracoccus denitrificans* and other hydrogen bacteria. FEMS Microbiol Lett 10:209–212

Gorlenko VM, Bonch-Osmolovskaya EA, Kompantseva EI, Starynin DA (1987) Differentiation of microbial communities in connection with a change in the physicochemical conditions in (a) thermophile spring (translation). Mikrobiologiya 56:314–322

Hole H, Grace D (1987) Polyglucose synthesis in *Chloroflexus aurantiacus* studied by ^{13}C-NMR. Arch Microbiol 148:292–297

Huss VAR, Festl H, Schleifer KH (1983) Studies on the spectrometric determination of DNA hybridization from renaturation rates. J Syst Appl Microbiol 4:184–192

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Muntu HN (ed) Mammalian protein metabolism. Academic Press, New York, pp 21–132

Kieft TL, Fredrickson JK, Onstott TC, Gorby YA, Kostandarithes HM, Bailey TJ, Kennedy DW, Li SW, Plymale AE, Spadoni CM, Gray MS (1999) Dissimilatory reduction of Fe(III) and other electron acceptors by a *Thermus* isolate. Appl Environ Microbiol 65:1214–1221

Kristjansson JK, Ingason A, Alfredsson GA (1985) Isolation of thermophilic obligately autotrophic hydrogen-oxidizing bacteria, similar to *Hydrogenobacter thermophilus* from Icelandic hot springs. Arch Microbiol 140:321–325

Kristjansson JK, Hjorleifsdottir S, Marteinsson VT, Alfredsson GA (1994) *Thermus scotoductus,* sp. nov., a pigment-producing thermophilic bacterium from hot tap water in Iceland and including *Thermus* sp. X-1. Syst Appl Microbiol 17:44–50

Maidak BL, Cole JR, Parker CT Jr, Garrity GM, Larsen N, Li B, Lilburn TG, McCaughey MJ, Olsen GJ, Overbeek R, Pramanik S, Schmidt TM, Tiedje JM, Woese CR (1999) A new version of the RDP (Ribosomal Database Project). Nucleic Acids Res 27:171–173

Manchenko GP (1994) Handbook of detection of enzymes on electrophoretic gels. CRC Press, Boca Raton

Mathews CK, van Holde KE (1990) Biochemistry. Benjamin/Cummings, Redwood City

Mesbah M, Premachandran U, Whitman W (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. Int J Syst Bacteriol 39:159–167

Murray RGE, Doetsch RN, Robinow CF (1994) Determinative and cytological light microscopy. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 21–41

Nelson DC, Castenholz RW (1981) Use of reduced sulfur compounds by *Beggiatoa* sp. J Bacteriol 147:140–154

Odintsova EV, Jannasch HW, Mamone JA, Langworthy TA (1996) *Thermothrix azorensis* sp. nov., an obligately chemolithoautotrophic, sulfur-oxidizing, thermophilic bacterium. Int J Syst Bacteriol 46:422–428

Petursdottir SK, Kristjansson JK (1996) The relationship between physical and chemical conditions and low microbial diversity in the Blue Lagoon geothermal lake in Iceland. FEMS Microbiol Ecol 19:39–45

Petursdottir SK, Kristjansson JK (1997) *Silicibacter lacuscaerulensis* gen. nov., sp. nov., a mesophilic moderately halophilic bacterium characteristic of the Blue Lagoon geothermal lake in Iceland. Extremophiles 1:94–99

Pitulle C, Yang Y, Marchiani M, Moore ERB, Siefert JL, Aragno M, Jurtshuk P Jr, Fox GE (1994) Phylogenetic position of the genus *Hydrogenobacter*. Int J Syst Bacteriol 44:620–626

Reasoner DJ, Geldreich EE (1985) A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49:1–7

Schenk A, Aragno M (1979) *Bacillus schlegelii*, a new species of thermophilic, facultatively chemolithoautotrophic bacterium oxidizing molecular hydrogen. J Gen Microbiol 115:333–341

Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS (1986) Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 51:873–884

Sirevaag R (1992) Thermophilic phototrophs. In: Kristjansson JK (ed) Thermophilic bacteria. CRC Press, Boca Raton, pp 195–219

Skirnisdottir S, Hreggvidsson GO, Hjorleifsdottir S, Marteinsson VT, Petursdottir SK, Holst O, Kristjansson JK (2000) Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats. Appl Environ Microbiol 66:2835–2841

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 607–654

Tabatabai MA (1974) A rapid method for determination of sulfate in water samples. Environ Lett 7:237–243

Williams R, Sharp R (1995) The taxonomy and identification of *Thermus*. In: Sharp R, Williams R (eds) *Thermus* species. Plenum, New York, pp 1–42