***Desulfonispora thiosulfatigenes* gen. nov., sp. nov., a taurine-fermenting, thiosulfate-producing anaerobic bacterium**

Karin Denger,¹ Erko Stackebrandt² and Alasdair M. Cook¹

Author for correspondence: Alasdair M. Cook. Tel: +49 7531 88 4247. Fax: +49 7531 88 2966.
e-mail: alasdair.cook@uni-konstanz.de

¹ Fakultät für Biologie der
Universität, D-78457
Konstanz, Germany

² DSMZ – Deutsche
Sammlung von
Mikroorganismen und
Zellkulturen GmbH,
Mascheroder Weg 1b,
D-38124 Braunschweig,
Germany

Strain GKNTAU^T has been described as a bacterium able to ferment the organosulfonate taurine (2-aminoethanesulfonate) quantitatively to acetate, ammonia and thiosulfate, an unusual metabolic product. This novel fermentation has now also been observed in four independent isolates from two continents. All five organisms were strictly anaerobic, Gram-positive, motile, spore-forming bacteria. Enrichments with isethionate (2-hydroxyethanesulfonate) and cysteate (2-amino-3-sulfopropionate), in contrast, yielded bacteria that disproportionated the sulfonate to sulfate and sulfide. The phylogenetic location of the taurine fermenters was analysed on the basis of 16S rDNA sequences. Strain GKNTAU^T (= DSM 11270^T = ATCC 700533^T) is described as the type strain of a new genus and species, for which the name *Desulfonispora thiosulfatigenes* gen. nov., sp. nov. is proposed.

Keywords: *Desulfonispora thiosulfatigenes*, taurine, thiosulfate, sulfonate, anaerobic desulfonation

INTRODUCTION

The first fermentation of an organosulfonate compound (i.e. containing the stable C–SO₃[−] bond) was reported recently (Denger *et al.*, 1997b; Cook *et al.*, 1998) and involved the major mammalian solute taurine (2-aminoethanesulfonate) (cf. Huxtable, 1992). This novel anaerobic reaction by the bacterial strain GKNTAU^T yielded acetate, ammonia and thiosulfate as quantitative metabolic products. This type of fermentation, if widespread, was proposed as a potential environmental source of thiosulfate (Denger *et al.*, 1997b), whose other known source as an end product of a degradative pathway is *Aquamicrobium defluvii* (Bambauer *et al.*, 1998). Thiosulfate is otherwise known as a widely used electron acceptor or donor by many aerobic and anaerobic bacteria.

A second bacterial fermentative reaction with an organosulfonate, a disproportionation of e.g. cysteate (2-amino-3-sulfopropionate) to yield sulfate and sulfide (as well as acetate and ammonia), has also been

discovered (Laue *et al.*, 1997a). It is thus relevant to establish whether the reaction found in strain GKNTAU^T is widespread or limited to the inoculum that we used in the initial work.

On the basis of a partial 16S rDNA sequence (Denger *et al.*, 1997b), strain GKNTAU^T was described as representing a novel genus in the *Syntrophomonas* assemblage within the *Clostridium* subdivision of the Gram-positive bacteria. In this communication, we present data on the widespread occurrence of relatives of strain GKNTAU^T for which the name *Desulfonispora thiosulfatigenes* gen. nov., sp. nov. is proposed.

METHODS

Enrichment, isolation and culture conditions. Enrichment cultures were made under aseptic conditions. Strictly anoxic conditions, confirmed by the absence of colour in the presence of 2 µM resazurine, were maintained in bicarbonate-buffered, titanium(III) nitrilotriacetate-reduced mineral salts medium at pH 7.0, which contained a single sulfonate as the sole source of carbon and energy as described previously (Denger *et al.*, 1997b). Cultures were incubated with occasional shaking at 30 °C in the dark. Putative enrichments were subcultured four or five times before bacteria were isolated by the agar shake method (Pfennig, 1978).

Abbreviations: CoM, coenzyme M; CYS, cysteate; ISE, isethionate; LK, Lake Konstanz; PU, Pushchino; RZ, Radolfzell; SU, Sunnyvale; TAU, taurine. The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence from *Desulfonispora thiosulfatigenes* DSM 11270^T is Y18214.

Table 1. Evaluation of the enrichment cultures

All cultures were anoxic, as indicated by the colourless resazurine redox indicator. The abbreviations indicate where stable enrichment cultures were obtained; the coding is a combination of the inoculum and the substrate utilized: taurine (TAU); cysteate (CYS); isethionate (ISE). —, No growth after 7 weeks incubation.

Geographic origin of inoculum	Taurine	Cysteate	Isethionate	Coenzyme M
Sewage works, Radolfzell (RZ), Germany	RZ/TAU	—	—	—
Sewage works, Pushchino (PU), Russia	PU/TAU	PU/CYS	PU/ISE	—
Sewage works, Sunnyvale (SU), California, USA	SU/TAU	SU/CYS	SU/ISE	—
Pool, Arastradero Lake, California, USA	—	—	—	—
Creek, Ronda, Spain	—	—	—	—
Sediment of Lake Konstanz (LK), Germany	LK/TAU	—	—	—

The inocula were from six sources (Table 1). Three samples originated from anaerobic digesters integrated in communal sewage works in Germany (RZ), Russia (PU) and California (SU), while two samples came from small freshwater habitats in Spain and California. Sample LK was derived from a lake sediment under an 80 m water column. The sampling sites for strains RZ and LK were in the Rhine basin, upstream of the sewage works in Konstanz from which strain GKNTAU^T was isolated (strain LK) or from a separate section of Lake Konstanz (Radolfzell) where no mixing of water samples is likely (strain RZ). The freshwater samples, when used for enrichments, were no longer black (putative iron sulfide) but were brown. Strain GKNTAU^T (= DSM 11270^T = ATCC 700533^T) (Denger *et al.*, 1997b) was already under study in the laboratory.

Morphology and physiology. Purity, morphology, motility and spore formation were checked microscopically. The Gram reaction was assayed in the KOH test (Gregersen, 1978). Oxidase and catalase tests were carried out according to standard methods (Gerhardt *et al.*, 1994). Utilization of the bile acids taurochenodeoxycholate, taurodeoxycholate or taurocholate was tested with 3 mM substrate each plus 3 mM added after 3 d.

Analytical methods. Taurine was derivatized and determined by reverse-phase HPLC (Denger *et al.*, 1997a). Acetate was quantified by GC (Laue *et al.*, 1997b). Ammonia was measured using the Berthelot reaction (Gesellschaft Deutscher Chemiker, 1996). Thiosulfate and sulfate were quantified by ion chromatography (Denger *et al.*, 1997b), and sulfide was determined colorimetrically (Cline, 1969). Cytochromes in strain GKNTAU^T were assayed in soluble and membrane fractions obtained after disruption of cells in a French press and a number of centrifugation steps (Denger *et al.*, 1997b). Redox-difference spectra of dithionite-reduced minus air-oxidized, or minus CO-reduced, samples were recorded with a Uvicon 922 spectrophotometer. The concentration of cytochrome *b* was calculated from the absorbance difference $\Delta E_{561} - \Delta E_{575}$, assuming a $\Delta \epsilon_{561-575}$ of 26.2 mM⁻¹ cm⁻¹ (Kröger & Innerhofer, 1976). SDS-PAGE with 4% stacking gels and 10% separating gels was performed using a standard method (Laemmli, 1970) with Coomassie staining. Protein was assayed using a dye-binding method (Bradford, 1976). The G+C content of DNA was determined by HPLC in the DSMZ service laboratory.

16S rDNA sequencing Extraction of genomic DNA, PCR-mediated amplification of 16S rDNA, purification of the

PCR products, and sequence analysis were performed as described elsewhere (Rainey *et al.*, 1996). Sequence reactions were electrophoresed using the Applied Biosystems 373A DNA sequencer. The sequence was aligned manually to 16S rRNA gene sequences of representative micro-organisms belonging to the domain *Bacteria* using the alignment editor ae2 (Maidak *et al.*, 1996). A phylogenetic tree was generated using the algorithm of De Soete (1983) – accession numbers are given in Fig. 3.

RESULTS AND DISCUSSION

Enrichment and isolation

Anoxic enrichment cultures were set up with 10 mM organosulfonate as the sole source of carbon and energy for six different inocula (Table 1). After incubation for 1 week, the first cultures developed turbidity, and bacteria could be observed microscopically. In total, eight enrichment cultures were obtained. All cultures utilizing taurine grew in 18–48 h when transferred regularly; the other cultures required about 5 d. After four to five transfers in homologous medium, each culture was dominated by a single organism. Thus it was easy to obtain bacteria, which ferment the natural products taurine (four enrichments), cysteate (two enrichments) and isethionate (2-hydroxyethanesulfonate; two enrichments) from inocula from different continents. Not all of the enrichments produced all of the end products found in samples from Sunnyvale and Pushchino (Table 1). The widespread coenzyme M was not dissimilated in any anaerobic organism tested (Lie *et al.*, 1996; Denger *et al.*, 1997a; Laue *et al.*, 1997b).

The fate of the sulfonate moiety was examined in each of the eight enrichments. The isethionate-utilizing and the cysteate-utilizing cultures produced a mixture of sulfide and sulfate, which is analogous to the end products of *Desulfovibrio* sp. strain GRZCYSA (Laue *et al.*, 1997a). While the isethionate-utilizing strains were vibrioform, the cysteate-utilizing cultures were spirilliform or rods. No spores were detected in the latter cultures.

Fig. 1. Phase-contrast photomicrograph of strain GKNTAU^T after growth in taurine mineral salts medium. Bar, 10 µm.

All of the four cultures that utilized taurine produced thiosulfate. Thus, as in our previous work on GKNTAU^T (Denger *et al.*, 1997b), we find only two patterns of energy conservation in the fermentation of organosulfonates, i.e. the formation of thiosulfate from sulfite in taurine metabolism (analogous to strain GKNTAU^T) and the disproportionation of putative sulfite to sulfate and sulfide in the metabolism of cysteate and isethionate (analogous to strain GRZCYSA).

A single organism was isolated from each of the taurine enrichment cultures and each isolate was a motile, spore-forming rod similar to strain GKNTAU^T. The organisms were 2–5 × 0.7–1.0 µm in size and formed subterminal spores. A phase-contrast photomicrograph of strain GKNTAU^T is shown in Fig. 1. The isolates were Gram-positive and oxidase-negative. Most were catalase-negative, except for strain SU/TAU. The pattern of proteins from crude extracts of all five strains after separation on SDS-PAGE was almost indistinguishable (not shown). The fermentation products from taurine were always equimolar amounts of acetate and ammonia together with 0.5 mol thiosulfate, as found with strain GKNTAU^T (Denger *et al.*, 1997b), so we consider that the same formal dissimilatory mass balance applies:

Neither sulfide nor sulfate was found in these cultures (as in *Desulfovibrio* sp. strain GRZCYSA; Laue *et al.*, 1997a). Thus all isolates that ferment taurine use the same overall pathway. None of these isolates grew with the three naturally occurring organosulfonates (cysteate, isethionate and coenzyme M).

Physiological properties of strain GKNTAU^T

This bacterium has become specialized for taurine fermentation, as none of the 36 carbon substrates tested supported growth (Denger *et al.*, 1997b). Bile

Fig. 2. Redox-difference spectrum of the membrane fraction of strain GKNTAU^T showing the spectrum of cytochrome *b*. The protein concentration was 1.3 mg ml⁻¹.

acids, e.g. taurochenodeoxycholate, taurodeoxycholate or taurocholate, demonstrated to be utilized by the taurine-reducing bacterium *Bilophila wadsworthia* RZATAU (Schumacher *et al.*, 1996), did not support growth. Sulfoacetate, 3-aminopropanesulfonate and β-alanine (each at 20 mM) were not utilized. Complex media, 0.2% (w/v) tryptone and 0.1% (w/v) yeast extract also failed to support growth of strain GKNTAU^T.

As it can be assumed that conservation of energy coupled to the formation of thiosulfate involves electron transport, the reddish-brown cell-free extracts of strain GKNTAU^T were tested for the presence of cytochromes. Absorption bands at 428, 530 and 559 nm (Fig. 2) were detected in both soluble and membrane fractions, which indicated the presence of *b*-type cytochromes (Voet & Voet, 1992). The content of cytochrome *b* in the soluble fraction was calculated to be about 0.08 µmol (g protein)⁻¹, while the level of cytochrome *b* in the membrane was about 0.6 µmol (g protein)⁻¹. Thus we attributed membrane-bound cytochrome *b* to a putative electron-transport chain involved in generating thiosulfate.

The source of the sulfur atoms in the thiosulfate synthesized by strain GKNTAU^T is sulfite (Denger *et al.*, 1997b), and a sulfite reductase was proposed to be involved in the pathway leading to thiosulfate (Denger *et al.*, 1997b). Difference spectra obtained in this study gave no support for the presence of desulforubidin (Lien & Beeder, 1997) or desulfofusicidin (Hatchikian & Zeikus, 1983). 'Shoulders' in the UV-visible spectrum of the oxidized form at 383 and 580 nm (literature values 383 and 581 nm), however, were preliminary evidence for a P582 sulfite reductase (Trudinger, 1970). This assumption was supported by oxidized minus dithionite-reduced difference spectra with minima at 446 and 580 nm and maxima at 558 and 622 nm (literature values 450, 581, 557 and 621 nm), and by characteristic difference spectra with CO (a minimum

Fig. 3. Phylogenetic dendrogram based on 16S rDNA sequence similarities between strain GKNTAU^T and the nearest related taxa (De Soete, 1983). Bar, 10% sequence similarity. *, Sequences derived from the Ribosomal Database Project (Maidak et al., 1997).

at 432 nm and a maximum at 594 nm (literature values 432 and 596 nm); cytochrome *b* interfered at other key parts of the spectrum.

Phylogenetic analysis

The almost complete 16S rDNA sequence of strain GKNTAU^T consisting of 1507 nucleotides was compared with currently available sequences of representative prokaryotes belonging to the domain *Bacteria*. Strain GKNTAU^T belongs to the *Clostridium* subdivision of the Gram-positive bacteria, but shows less than 88% sequence similarity with any of the deposited sequences. The highest values of 86–88% similarity are found with sequences from members of the genera *Desulfotomaculum*, *Desulfitobacterium*, *Desulfosporosinus* and *Peptococcus*. A relationship dendrogram (De Soete, 1983) demonstrates the position of strain GKNTAU^T next to its nearest neighbours (Fig. 3). Bootstrap values are low for the majority of branch points, including that leading to strain GKNTAU^T and *Peptococcus niger*. The low level of relatedness to the most closely related species and the lack of physiologically similar organisms in the phylogenetic neighbourhood led us to the conclusion that the new thiosulfate-producing strain represents a new genus and species; the name *Desulfonispora thiosulfatigenes* gen. nov., sp. nov., with strain GKNTAU^T (= DSM 11270^T = ATCC 700533^T) as the type strain, is proposed. The strains RZ/TAU, PU/TAU, SU/TAU and LK/TAU are affiliated to this species because of the high similarities in physiological and morphological properties.

The G+C content of strain GKNTAU^T was determined to be 52 mol%. This value falls within the

range of the *Clostridium* subdivision (21–54 mol%; Hippe et al., 1992), which is widely considered to have a low G+C content.

Description of *Desulfonispora thiosulfatigenes* gen. nov., sp. nov.

Desulfonispora thiosulfatigenes [De.sul.fo.ni.spo'ra. M.L. pref. *desulfono* desulfonating; L. fem. n. *spora* spore; M.L. fem. n. *Desulfonispora* desulfonating spore (-former); thi.o.sul.fa.ti'ge.nes. M. L. n. *thiosulfas* thiosulfate; M.L. suff. *genes* -producing; M.L. part. adj. *thiosulfatigenes* thiosulfate-producing].

Rod-shaped bacteria, 2–5 × 0.7–1.0 µm in size, motile, with subterminal spores. Oxidase- and catalase-negative. High cytochrome *b* level in membrane fractions. Mesophilic. Growing only with taurine (out of 41 substrates tested), producing acetate, ammonia and thiosulfate. Neither sulfate, sulfite nor nitrate is reduced. In terms of phylogeny, the type species is a member of the *Clostridium* subline of descent, being remotely related to *Peptococcus*, *Desulfotomaculum*, *Desulfitobacterium* and *Desulfosporosinus*. The G+C content is 52 mol%. Strain GKNTAU^T is the type strain and is deposited with the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) under DSM 11270^T and with the American Type Culture Collection under ATCC 700533^T.

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