Genome-enabled analysis of the utilization of taurine as sole source of carbon or of nitrogen by *Rhodobacter sphaeroides* 2.4.1

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A degradative pathway for taurine (2-aminoethanesulfonate) in *Rhodobacter sphaeroides* 2.4.1 was proposed by Brüggemann *et al.* (2004) (*Microbiology* **150**, 805–816) on the basis of a partial genome sequence. In the present study, *R. sphaeroides* 2.4.1 was found to grow exponentially with taurine as the sole source of carbon and energy for growth. When taurine was the sole source of nitrogen in succinate-salts medium, the taurine was rapidly degraded, and most of the organic nitrogen was excreted as the ammonium ion, which was then utilized for growth. Most of the enzymes involved in dissimilation, taurine dehydrogenase (TDH), sulfoacetaldehyde acetyltransferase (Xsc) and phosphate acetyltransferase (Pta), were found to be inducible, and evidence for transcription of the corresponding genes (*tauXY*, *xsc* and *pta*), as well as of *tauKLM*, encoding the postulated TRAP transporter for taurine, and of *tauZ*, encoding the sulfate exporter, was obtained by reverse-transcription PCR. An additional branch of the pathway, observed by Novak *et al.* (2004) (*Microbiology* **150**, 1881–1891) in *R. sphaeroides* TAU3, involves taurine:pyruvate aminotransferase (Tpa) and a presumptive ABC transporter (NsbABC). No evidence for a significant role of this pathway, or of the corresponding alanine dehydrogenase (Ald), was obtained for *R. sphaeroides* 2.4.1. The anaplerotic pathway needed under these conditions in *R. sphaeroides* 2.4.1 seems to involve malyl-CoA lyase, which was synthesized inducibly, and not malate synthase (GlcB), whose presumed gene was not transcribed under these conditions.

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INTRODUCTION

Taurine (2-aminoethanesulfonate) (Fig. 1a) is 'a phylogenetically ancient compound with a disjunct distribution in the biosphere' (Huxtable, 1992). Absent in plants, it is a major organic solute in mammals [1 g (kg wet wt)⁻¹], which excrete it (largely in urine), and it is found free or derivatized in all vertebrates, many marine creatures, algae and bacteria (e.g. Allen & Garrett, 1971; Vollrath *et al.*, 1990; Huxtable, 1992; Yin *et al.*, 2000; Abraham *et al.*, 2004). Spiders secrete molar concentrations of *N*-acetyltaurine on their webs (Vollrath *et al.*, 1990). The widespread supply of extracellular (and intracellular) taurine is presumed to explain the large number of terrestrial and marine bacteria able to utilize the compound as sole source of carbon for aerobic growth, as a source or sink of electrons in respirations, and in fermentations or in photoheterotrophic growth (e.g. Lie *et al.*, 1998; Cook & Denger, 2002; Denger *et al.*, 2004a; Novak *et al.*, 2004). The compound can also serve as a sole source of sulfur for growth (e.g. Kertesz, 2000), and a range

of phenomena can be involved when organisms utilize the compound as a sole source of nitrogen; these include deamination without desulfonation, not always concomitant with substrate utilization (Denger *et al.*, 2004b; Styp von Rekowski *et al.*, 2005; Weinitschke *et al.*, 2005), and utilization concomitant with taurine carbon (Denger *et al.*, 2004a). Two general pathways for the dissimilation of taurine have been hypothesized (Cook & Denger, 2006), one of which has received significant experimental support in *Silicibacter pomeroyi* DSS-3, especially for the genes encoding an ATP-binding cassette transporter (TauABC), taurine:pyruvate aminotransferase (Tpa) and alanine dehydrogenase (Ald) (analogous to Fig. 1a, bottom left), as well as sulfoacetaldehyde acetyltransferase (Xsc) and phosphate acetyltransferase (Pta) (Gorzynska *et al.*, 2006).

The second hypothesis (Fig. 1a, b, Table 1) was first formulated from data in the partial genome sequence of the facultative phototroph *Rhodobacter sphaeroides* 2.4.1 (Brüggemann *et al.*, 2004), which now serves as a model organism that represents several genomes or sequences of clusters of taurine-related genes. The hypothesis involves in part a gene cluster now known to be encoded on plasmid B of *R. sphaeroides* 2.4.1 (Fig. 1b). The cluster contains the genes for a putative regulator (*tauR*) and a tripartite

Abbreviations: Ald, alanine dehydrogenase; Pta, phosphate acetyltransferase; RT, reverse transcription; TDH, taurine dehydrogenase; Tpa, taurine:pyruvate aminotransferase; TRAP, tripartite ATP-independent; Xsc, sulfoacetaldehyde acetyltransferase.

Table 1. Annotations of portions of the genome of *R. sphaeroides*

Rsph no.	Gene	Enzyme	EC (TC) no.
RSP_4017*	<i>tauR</i> †	Transcriptional regulator	
RSP_4016*	<i>tauK</i> †	Substrate binding protein	TC 2.A.56.4.1
RSP_4015*	<i>tauL</i> †	Small membrane component	TC 2.A.56.4.1
RSP_4014*	<i>tauM</i> †	Large membrane component	TC 2.A.56.4.1
RSP_4013*	<i>tauX</i> †	Small subunit of taurine dehydrogenase	EC 1.4.2.–
RSP_4012*	<i>tauY</i> †	Large subunit of taurine dehydrogenase	EC 1.4.2.–
RSP_4011*	<i>xsc</i> †	Sulfoacetaldehyde acetyltransferase	EC 2.3.3.15
RSP_4010*	<i>tauZ</i> †	Sulfate exporter	TC 9.B.63.1.1
RSP_4009*	<i>pta</i> ‡	Phosphate acetyltransferase	EC 2.3.1.8
RSP_0888§	<i>amtB</i> ‡	Ammonium/methylammonium transport	TC 2.A.49.1.–
RSP_0940§	<i>nsbA</i> ‡	Nitrate/sulfonate/bicarbonate ABC transporter	TC 3.A.1.–.–
RSP_0941§	<i>nsbB</i> ‡	Nitrate/sulfonate/bicarbonate ABC transporter	TC 3.A.1.–.–
RSP_0942§	<i>nsbC</i> ‡	Nitrate/sulfonate/bicarbonate ABC transporter	TC 3.A.1.–.–
RSP_0943§	<i>tpa</i> †	(Taurine aminotransferase) COG0161	(EC 2.6.1.–)
RSP_0723§	<i>ald</i> ‡	Alanine dehydrogenase	EC 1.4.1.1
None		Isocitrate lyase	EC 4.1.3.1
RSP_1980§	<i>glcB</i> ‡	(Malate synthase)	(EC 2.3.3.9)
RSP_1771§	<i>mclI</i>	Malyl-CoA lyase	EC 4.1.3.24
Unknown		Malyl-CoA hydrolase	EC 3.1.2.– (18?)
Unknown		Sulfite dehydrogenase	EC 1.8.2.1

*Located on plasmid B.

†Annotated by the present authors.

‡Annotated by JGI.

§Located on chromosome 1.

||Annotated by Meister *et al.* (2005).

We now report that the degradative pathway for taurine as sole source of carbon or nitrogen for growth for *R. sphaeroides* 2.4.1 involves the TRAP transporter and TDH, whereas the *tpa* orthologue is not relevant.

METHODS

Bacteria, media and culture conditions. *R. sphaeroides* 2.4.1^T, whose genome has been sequenced, was kindly provided by S. Kaplan, University of Texas, Houston, TX, USA. Cultures of *R. sphaeroides* 2.4.1 were grown at 30 °C in the dark in a modification of Sistrom's (Sistrom, 1962) minimal medium. It contained 50 mM potassium phosphate buffer, pH 7.2, 0.25 mM MgSO₄, 9 mM NaCl, 1.2 mM MgCl₂, 0.2 mM CaCl₂, trace elements and a vitamin solution (Thurnheer *et al.*, 1986). Different combinations of sources of carbon and nitrogen for growth were used: 10 mM taurine as sole and limiting source of carbon with 2 mM NH₄Cl [taurine(carbon)-ammonium medium]; 2 mM taurine as nitrogen source (a growth-limiting concentration) with 9 mM succinate [taurine(nitrogen)-succinate medium]; 2 mM NH₄Cl with 9 mM succinate (succinate-ammonium medium). Purity was checked by streaking on Luria-Bertani agar plates and observing homogeneous, red-coloured colonies.

Precultures (5 ml) were grown in 30 ml screw-cap tubes in a roller. Growth experiments, or cultures for harvesting and preliminary enzyme assays, were done with 50 ml cultures in 300 ml Erlenmeyer flasks on a rotary shaker. Samples were taken in intervals to measure OD₅₈₀, to quantify protein (OD₅₈₀ 1≈205 mg protein l⁻¹) and to

determine the concentrations of substrate and products. Cells for the preparation of total RNA were grown in the required selective medium and harvested in the mid-exponential phase of growth (OD₅₈₀ 0.3–0.7) by centrifugation at 5000 g; RNA was extracted immediately. Storage of intact cells at –70 °C before RNA extraction resulted in complete loss of mRNA.

Preparation of cells grown with taurine as a nitrogen source for RNA extraction or enzyme determination involved a growth phase in taurine(nitrogen)-succinate-salts medium followed by renewed induction by addition of taurine (to 2 mM) 3–10 h before harvesting. Cells for enzyme purification were grown with taurine as a carbon source at the 1-litre scale in 5-litre Erlenmeyer flasks. Disruption of cells and the preparation of crude extracts, a membrane fraction and a soluble fraction were as described elsewhere (Weinitschke *et al.*, 2006).

Enzyme assays and protein separation. Taurine dehydrogenase (TDH) was measured photometrically with dichlorophenol indophenol as the electron acceptor as described previously (Brüggemann *et al.*, 2004). Taurine:pyruvate aminotransferase (Tpa) was assayed discontinuously as the pyruvate-dependent disappearance of taurine concomitant with the formation of alanine (Brüggemann *et al.*, 2004). Alanine dehydrogenase (Ald) was routinely measured photometrically as the reduction of NAD⁺ (Laue & Cook, 2000a); *S. pomeroyi* DSS-3 (Denger *et al.*, 2006) served as a positive control. Sulfoacetaldehyde acetyltransferase (Xsc) was assayed by GC as the ThDP- and phosphate-dependent release of acetate after acidification to hydrolyse the acetyl phosphate formed, or assayed as the formation of sulfite (Ruff *et al.*, 2003). Phosphate acetyltransferase (Pta) was assayed photometrically as the HS-CoA-dependent formation of acetyl-CoA (Bergmeyer *et al.*, 1983). Sulfite dehydrogenase

was assayed with potassium ferricyanide or beef cytochrome *c* (Reichenbecher *et al.*, 1999) as the electron acceptor, and *Sinorhizobium meliloti* Rm1021 (Brüggemann *et al.*, 2004) and *Delftia acidovorans* NAT (Mayer *et al.*, 2006) were used as positive controls. Other putative electron acceptors, O₂, NAD(P), FAD and nitrate, were tested, as was the possibility of an AMP- or ADP-dependent oxidation with ferricyanide or cytochrome *c* (Hagen & Nelson, 1997). Isocitrate lyase and malate synthase were assayed as described by Dixon & Kornberg (1959), and *D. acidovorans* NAT (Mayer *et al.*, 2006) served as positive control. Maly-CoA lyase was assayed as described elsewhere (Meister *et al.*, 2005). Anion-exchange and hydrophobic interaction chromatography were done with soluble fractions free of nucleic acids as described elsewhere (Ruff *et al.*, 2003; Rein *et al.*, 2005).

Analytical methods. Growth was followed as turbidity (OD₅₈₀) and quantified as protein in a Lowry-type reaction (Cook & Hütter, 1981). Taurine and alanine were derivatized with dinitrofluorobenzene and subjected to separation by HPLC (Denger *et al.*, 1997). Sulfite was quantified as the fuchsin derivative as described elsewhere (Denger *et al.*, 2001). Sulfate was determined turbidimetrically as a suspension of BaSO₄ (Sörbo, 1987). Ammonium ion was assayed colorimetrically by the Berthelot reaction (Gesellschaft Deutscher Chemiker, 1996). Protein in extracts was assayed by protein-dye binding (Bradford, 1976). Denatured proteins were separated in 12 % SDS-PAGE gels and stained with Coomassie Brilliant Blue R250 (Laemmli, 1970).

Molecular methods. Oligonucleotides were synthesized by Microsynth (Balgach, Switzerland). *Taq* DNA polymerase and M-MuLV reverse transcriptase were from MBI Fermentas and they were used as specified by the supplier. Chromosomal DNA was isolated from bacteria as described by Desomer *et al.* (1991). Total RNA was isolated using the EZNA bacterial RNA kit (Peglab

Biotechnologie), and contaminant DNA was removed with RNase-free DNase (Qiagen). The RNA was tested for residual DNA before reverse transcription (RT) by PCR using the primer set RsXscF-RsXscR. The reverse PCR primers listed in Table 2 were used for RT-PCR reactions. Subsequent PCR reactions were done as described previously (Innis *et al.*, 1990) using chromosomal DNA of *R. sphaeroides* 2.4.1 as a positive control. PCR products were visualized on 1 % or 1.5 % agarose gels according to standard methods (Sambrook *et al.*, 1989). The GeneRuler DNA ladder mix (MBI Fermentas) was used as molecular marker.

Software for DNA analyses. Sequence analysis of the *R. sphaeroides* 2.4.1 genome (accession nos CP000143 to CP000147 and DQ232586 to DQ232587) were done using the BLAST algorithm (Altschul *et al.*, 1997) at NCBI. The Lasergene package (DNASStar) was used for routine DNA analyses *in silico*. Primers for RT and PCR were designed using the program Amplify (version 1.2).

RESULTS

Taurine as a carbon source for aerobic growth of *R. sphaeroides* 2.4.1

R. sphaeroides 2.4.1 grew in 11 mM taurine(carbon)-ammonium medium with a specific growth rate (μ) of 0.13 h⁻¹ (Fig. 2a). The utilization of taurine and the excretion of both ammonium and sulfate ions were concomitant with growth (Fig. 2b). The yield of sulfate at the end of growth was 11 mM. No sulfite was detected. A net increase of about 9 mM ammonium ion was found in the medium at the end of growth, and we calculated there to be about 2 mM combined nitrogen in cell material in the

Table 2. Primers used in this study

Target gene	Primer name	Sequence (5–3')	Reference
<i>xsc</i>	RsXscF	GAACATGCGTTCGGGATCAT	This study
	RsXscR	GCCCTGCCCGATGGTCTTGTT	
<i>tauXY</i>	RsTauXF	ACCCGCGGCGTGAGCCAGTGGAC	This study
	RsTauYR	CGATCACCGCCACATCCACAT	
<i>tauK</i>	RsTauKF	ATGATCGAGAAGACGGTCGAGGAT	This study
	RsTauKR	AGCGCAGCTCGGTGACATATT	
<i>tauL</i>	RsTauLF	CTGTTCTGGATCGGCTTCAACCT	This study
	RsTauLR	CTCGTCCTCGGCCAGTTCCATCA	
<i>tauM</i>	RsTauMF	CCATGGTCTATCTGGGCGACAA	This study
	RsTauMR	CGAGACCGACTGGAAGGGACCGT	
<i>tpa</i>	RsTpaF	GAAC TTCGGCGTGAAACCCGATT	This study
	RsTpaR	CGGCCCGCAGAACAGACCCCT	
<i>tauZ</i>	RsTauZF	GCGAGACGGCGACGCTCGTCAA	This study
	RsTauZR	AGACGCCGAGGAAGATCGTTTC	
<i>pta</i>	RsPtaF	GGCCCGCGCCACCCGCGCCATA	This study
	RsPtaR	CAGGCGAGCATGAGAAAGAAGCTC	
<i>glcB</i>	RsMSF	GCGCTATGCGCTGAACGCGGCCAA	This study
	RsMSR	CAGGCCCGCGTGGCCCGCAAAT	
<i>amtB</i>	RsAmtBF	TCGTGACCCGGTGATGGACAA	This study
	RsAmtBR	TCTGGAAGCAGATGAACAGATA	
16S-rRNA	16S-27F	CAGAGTTTGATCCTGGCTCAG	Weisburg <i>et al.</i> (1991)
	16S-533R	TTACCGCGGCTGCTGGCAC

Fig. 2. (a) Growth of *R. sphaeroides* 2.4.1 in taurine(carbon)-ammonium medium. (b) Concentrations of substrate and products as a function of the protein concentration: ▲, taurine; ■, ammonium ion; ○, sulfate ion; □, sulfite ion.

medium. The molar growth yield, $6 \text{ g protein (mol C)}^{-1}$, represented quantitative utilization of the carbon source (Cook, 1987). The data were interpreted to represent a complete mass balance for the carbon, nitrogen and sulfur moieties in taurine.

Taurine as a sole source of nitrogen for *R. sphaeroides* 2.4.1

R. sphaeroides 2.4.1 grew biphasically in 2 mM taurine(nitrogen)-succinate medium (Fig. 3a). There was no growth in the absence of combined nitrogen. Taurine was rapidly metabolized, and it disappeared before 25 % of the total growth occurred (Fig. 3b). Over the same period, 2 mM sulfate was formed, so there was mass balance for sulfur; no sulfite was detected (Fig. 3b). Taurine nitrogen was released as the ammonium ion, and the organism then grew with this ammonium; growth was then concomitant with ammonium utilization (Fig. 3b). The overall molar growth yield was $70 \text{ g protein (mol taurine-N)}^{-1}$. The same growth yield [about $70 \text{ g protein (mol N)}^{-1}$] was observed in

Fig. 3. (a) Growth of *R. sphaeroides* 2.4.1 in taurine(nitrogen)-succinate medium. (b) Concentrations of nitrogen substrate and products as a function of the protein concentration: ▲, taurine; ■, ammonium ion; ○, sulfate ion; □, sulfite ion.

succinate-ammonium medium (not shown), which indicated quantitative assimilation of nitrogen into cell material (e.g. Cook, 1987). The specific growth rate in the presence of taurine was 0.08 h^{-1} ; a lower growth rate (maximally 0.03 h^{-1}) was observed when the excreted ammonium ion was the sole source of nitrogen (Fig. 3).

Enzyme activities and gene transcription

The first interactions of cell and substrate in a degradative pathway are the vectorial steps in transport across the cell membrane. Taurine uptake is postulated to be catalysed by an inducible TRAP transporter, TauKLM (Brüggemann *et al.*, 2004). No transcript of any of the corresponding genes was detected in succinate-ammonium-grown cells (Table 3). An amplicon of each of these genes, but especially of *tauL* and *tauM*, was obtained from taurine(carbon)-ammonium-grown cells (Table 3). Amplicons of *tauK*, *tauL* and *tauM* could also be detected in taurine(nitrogen)-succinate-grown cells (Table 3). This is the first direct evidence to show that the *tauKLM* genes are inducibly

Table 3. Specific activities of enzymes and transcription of genes under different conditions of growth of *R. sphaeroides* 2.4.1

Gaps in the table indicate that there is no assay available (e.g. transport) or not applicable (e.g. no gene to express). ND, Not determined; bdl, below detection limit. The limits of detection for TDH, Sor and Ald were 0.02 mkat (kg protein)⁻¹; for Xsc 0.3 mkat (kg protein)⁻¹, and for Pta, Icl and maly-CoA lyase, 0.1 mkat (kg protein)⁻¹.

Enzyme/transporter	Succinate/NH ₄ ⁺		Taurine-(C)/NH ₄ ⁺		Taurine-(N)/succinate	
	Enz*	RNA†	Enz	RNA	Enz	RNA
TauK		—		tr		tr
TauL		—		+		+
TauM		—		+		+
Taurine dehydrogenase (TDH, <i>tauXY</i>)	bdl	—	1.0	++	0.6	++
Taurine:pyruvate aminotransferase (Tpa)	1.3	+	0.2	+	0.3	+
Alanine dehydrogenase (Ald)	bdl	ND	bdl	ND	bdl	ND
Sulfoacetaldehyde acetyltransferase (Xsc)	bdl	—	4.2	++	3.3	++
TauZ		—		++		ND
Phosphate acetyltransferase (Pta)	bdl	—	6.0	+	0.4	ND
Sulfite dehydrogenase (Sor)	bdl		bdl		bdl	
Isocitrate lyase (Icl)	bdl		bdl		bdl	
Malate synthase	0.3	—	3.9	—	0.5	ND
Maly-CoA lyase	0.1	ND	0.5	ND	0.2	ND

*Enz, enzyme activity in mkat (kg protein)⁻¹.

†RNA, RNA transcript, the intensity of amplicons is scored as: —, absent; tr, trace; +, weak band; ++, strong band (see Fig. 4).

transcribed, and that their gene products are presumably relevant to taurine metabolism.

The activities of several enzymes were assayed in extracts of cells grown with either taurine or succinate as the sole source of carbon. TDH, Xsc and Pta were found in taurine(carbon)-ammonium-grown cells (Table 3) but not in succinate-ammonium-grown cells, so these enzymes were considered to be inducible. Inducible transcription of *xsc* and *pta* was detected in these cells (Table 3); inducible synthesis of TDH, Xsc and Pta, with the corresponding transcripts, was observed in taurine(nitrogen)-succinate-grown cells (Table 3). As in previous work (Brüggemann *et al.*, 2004; Weinitschke *et al.*, 2006), TDH was found to be associated with the particulate fraction of cell extracts, whereas Xsc and Pta were found in the soluble fraction. A transcript from the *tauXY* genes was detected in taurine(carbon)-ammonium-grown cells and in the taurine(nitrogen)-succinate-grown cells, but not in succinate-ammonium-grown cells (Table 3); the amplicon overlapped the *tauX* and *tauY* genes, so the signal indicates that these genes are co-transcribed. These data were considered to be further support for the postulate that *tauXY* encodes TDH (Brüggemann *et al.*, 2004; Weinitschke *et al.*, 2006).

Transamination of taurine was detected, but activity was higher in extracts of succinate-ammonium-grown cells than in extracts of taurine(carbon)-ammonium-grown or taurine(nitrogen)-succinate-grown cells (Table 3). Correspondingly, the transcript from the putative *tpa* gene was detected in cells from all cultures (Table 3). Further, no Ald activity was detected in extracts of cells

grown in the presence of taurine (Table 2), so negligible amounts of pyruvate could be regenerated from alanine. It was concluded that the putative Tpa and Ald made a negligible contribution to taurine dissimilation.

Sulfite dehydrogenase must have been present, because no sulfite was observed during growth (Figs 2b and 3b), but no enzyme activity was detected in cell extracts. The acceptors tested for the direct oxidation (Kappler & Dahl, 2001) were cytochrome *c* and ferricyanide, so neither the known sulfite dehydrogenase, SorAB (Kappler *et al.*, 2000), nor the cytochrome *c*-independent enzyme (Reichenbecher *et al.*, 1999; Gorzynska *et al.*, 2006; Weinitschke *et al.*, 2006), was detected. The acceptors tested for the AMP-dependent (indirect) pathway of sulfite oxidation (Hagen & Nelson, 1997; Kappler & Dahl, 2001) were ferricyanide and cytochrome *c*. The absence of SorAB (and SoxCD) could be anticipated from the genome sequence, and the indirect pathway is oxygen-sensitive (Schiffer *et al.*, 2006), so it is unclear whether the undefined ferricyanide-coupled enzyme (Reichenbecher *et al.*, 1999), or a novel enzyme, catalyses the oxidation of sulfite.

The product of sulfite dehydrogenase is sulfate, which is believed to be exported from the cell by TauZ (Rein *et al.*, 2005). A strong transcript of the *tauZ* gene was detected in taurine(carbon)-ammonium-grown cells, but not in succinate-ammonium-grown cells (Table 3). An amplicon overlapping the *tauZ* and *pta* genes was also obtained when using primer RsPtaR for RT (Fig. 4), so these neighbouring genes were co-transcribed. No amplicon was obtained for a transcript representing *tauX* to *xsc* (using primers

Fig. 4. Transcription and co-transcription of *tauZ* and *pta* from *R. sphaeroides* 2.4.1. The predicted sizes of the amplicons with the primer combinations used are: RsTauZF-RsTauZR, 344 bp; RsPtaF-RsPtaR, 410 bp; RsTauZF-RsPtaR, 822 bp. Lanes: M, GeneRuler DNA ladder mix; T, RNA from taurine(carbon)-ammonium-grown cells; S, RNA from succinate-ammonium-grown cells; +, positive control (genomic DNA); -, negative control (H₂O as template). Relevant marker sizes (in bp) are indicated on the left-hand and right-hand sides of the figure.

RsTauXF-RsXscR), or of a transcript representing *xsc* to *tauZ* (using primers RsXscF-RsTauZR); this suggests separate transcripts for *tauXY*, for *xsc* and for *tauZ-pta* in *R. sphaeroides* 2.4.1.

No activity of isocitrate lyase was detected in *R. sphaeroides* 2.4.1 (Table 3), as observed and cited by, for example, Filatova *et al.* (2005) and anticipated from the absence of the corresponding gene in the genome. The apparent activity of malate synthase was detected at a high level in taurine(carbon)-ammonium-grown cells, but at much lower levels in taurine(nitrogen)-succinate grown cells and in succinate-ammonium grown cells (Table 3). Malate synthase is one enzyme in Ivanovsky's anaplerotic pathway (e.g. Filatova *et al.*, 2005), but Meister *et al.* (2005) have shown this to result from the combined activity of malyl-CoA lyase (EC 4.1.3.24) and of a putative malyl-CoA hydrolase. We could confirm the presence of malyl-CoA lyase in crude extracts (Table 3) and detect a putative malyl-CoA hydrolase activity in fractions of the extract (not shown), analogous to the data of Meister *et al.* (2005). Correspondingly, the gene annotated to encode malate synthase, RSP_1980, was not transcribed under the conditions tested (Table 3).

Proteins in the extracts of cells grown in succinate-ammonium medium or in taurine(carbon)-ammonium medium were separated by SDS-PAGE (not shown). One inducible protein only could be observed in extracts of taurine(carbon)-ammonium-grown cells and taurine(nitrogen)-succinate-grown cells. The protein had a molecular mass of about 63 kDa, i.e. that of Xsc. Soluble extract of taurine(carbon)-ammonium-grown cells was subjected to anion-exchange chromatography and hydrophobic interaction chromatography, and the fraction containing Xsc activity, which was ThDP-dependent, was the essentially pure 63 kDa protein. The activity of Pta was lost rapidly and traces were found in many fractions from the anion

exchanger. A single fraction with Pta activity was obtained from soluble extract on the hydrophobic interaction column, but the losses in activity prevented both further chromatography, and useful analyses by SDS-PAGE.

DISCUSSION

The prediction that *R. sphaeroides* 2.4.1 would utilize taurine as the sole source of carbon for growth (Brüggemann *et al.*, 2004) has been confirmed, and growth was concomitant with substrate utilization (Fig. 2a, b). Similarly, the idea was confirmed that the organism would utilize taurine as a sole source of nitrogen for growth (Fig. 3a, b). The assimilation of taurine-nitrogen sometimes involves utilization of the compound concomitant with growth, as found in *Acinetobacter calcoaceticus* SW1, *Alcaligenes faecalis* MT1 and *Rhodococcus opacus* ISO-5 (Denger *et al.*, 2004a; Weinitschke *et al.*, 2005, 2006), and sometimes with excretion and subsequent utilization of the ammonium ion as in *Rhodopseudomonas palustris* CGA009 (Denger *et al.*, 2004b), as shown in Fig. 3(b). When the organism can utilize taurine as sole source of both carbon and nitrogen, the same pathway is obviously involved, via Tpa (and Ald) in *R. opacus* ISO-5 (Denger *et al.*, 2004a) and via TDH in *R. sphaeroides* 2.4.1 (Table 3). We have no explanation for the excretion or non-excretion of the ammonium ion during growth of different organisms with limiting amounts of taurine.

One key, novel aspect of the degradative pathway proposed by Brüggemann *et al.* (2004) is the TRAP transporter, TauKLM. We now present the first experimental evidence (Table 3) that the genes are transcribed, and presumably translated. The assumption of function is the sequence similarity to TRAP transporters for dicarboxylates (DctPQM) from *Rhodobacter capsulatus* (e.g. Forward *et al.*, 1997): TauK and DctP share 22 % identity. Gene orthologues of *tauKLM* have now been found in the

genomes of several organisms which contain the *xsc* gene and do, or can be expected to, dissimilate taurine [two other members of the Rhodobacterales (e.g. Brüggemann *et al.*, 2004), *Desulfotalea psychrophila* LSv54 (R. Rabus, personal communication) and three untested γ -Proteobacteria (Vibrionales)]. Transport of taurine via a TRAP transporter thus seems to be widespread.

The degradative pathway for taurine via TDH (Brüggemann *et al.*, 2004) was detected (Table 3). There was no direct proof that *tauXY* was involved in the pathway, but the inducible co-transcription of the two genes supports a role for the gene products, which we consider to be TDH. Heterologous expression of the *tauXY* genes has not yet been successful (T. H. M. Smits, unpublished), and solubilization of the membrane fraction led to loss of enzyme activity (K. Denger, unpublished). The identity of the cytochrome *c* which accepts electrons from TDH, is also unknown.

The prediction of a role for Tpa in taurine dissimilation by *R. sphaeroides* 2.4.1, from both sequence analysis (see Introduction) and enzyme assays in *R. sphaeroides* Tau3 (Novak *et al.*, 2004), was not confirmed. Some aminotransferase activity could, indeed, be detected, but at lower levels than in succinate-ammonium-grown cells (Table 3). We presume the taurine: pyruvate aminotransferase activity to represent the side reaction of a different aminotransferase. Work with the Tpa from *Bilophila wadsworthia* RZATAU showed that the sequences of some Tpa orthologues cluster together, whereas others are spread amongst class III aminotransferases of different or unknown functions (Laue *et al.*, 2006). The hypothetical ABC transporter ('NsbABC'; Fig. 1b, Table 3) encoded upstream of this transaminase is a member of a large group of uncharacterized ABC transporters closely related to TC 3.A.1.12/16/17.– which are unfortunately termed TauABC, identical to the TauABC transporters involved in taurine metabolism (TC 3.A.1.17.1) (Eichhorn *et al.*, 2000; Gorzynska *et al.*, 2006). TauA and NsbA share <20 % sequence identity. It is unlikely that 'NsbABC' (Table 3) plays any role in taurine metabolism in *R. sphaeroides* 2.4.1.

Xsc is the only enzyme in the pathway which has been regularly purified (Denger *et al.*, 2001, 2004a; Ruff *et al.*, 2003; Brüggemann *et al.*, 2004). In the present paper, the enzyme could be purified to near homogeneity in two steps. Indeed, the *xsc* gene is our marker for the presence of the degradation of a C₂-sulfonate in a genome (see Cook & Denger, 2002), and some 25 organisms have been confirmed to express the enzyme (e.g. Gorzynska *et al.*, 2006), or at least grow with a C₂-sulfonate (K. Denger, unpublished), and the number is steadily increasing.

Pta, in contrast, frequently presents a problem, because the enzyme is labile under our standard conditions (e.g. Weinitschke *et al.*, 2006), a phenomenon which seems to be widespread (Lawrence *et al.*, 2006). In the present study, the enzyme rapidly lost activity and could not be satisfactorily separated by anion-exchange chromatography.

Higher stability was attained for hydrophobic interaction chromatography, but the very low amount of partially purified protein did not allow an estimation of the molecular mass of the enzyme.

There will be several fates for the acetyl-CoA formed by Pta. A minor portion will flow to fatty acid synthesis. A major portion will be oxidized via the Krebs cycle, and a significant portion will flow through an anaplerotic sequence to maintain the supply of oxaloacetate for the Krebs cycle. The nature of this anaplerotic pathway is unclear, because *R. sphaeroides* has been known for some 40 years to lack one key enzyme of the glyoxylate shunt, isocitrate lyase. Ivanovsky's group, working in crude extracts, has postulated a citramalate cycle, which involves malate synthase (e.g. Filatova *et al.*, 2005). Fuchs' group has started to clarify this pathway by purifying enzymes, and a recent article shows that the apparent measurement of malate synthase represents the combined activity of malyl-CoA lyase and a malyl-CoA hydrolase (Meister *et al.*, 2005). Our data (e.g. Table 3) support this latter observation. Whatever the nature of the complete anaplerotic pathway (see Alber *et al.*, 2006), the apparent malate synthase in crude extract shows the cycle to be present at high levels in the taurine(carbon)-ammonium-grown cells, but only in traces in taurine(nitrogen)-succinate-grown cells (Table 3). Presumably, when taurine is supplying nitrogen in the presence of a C₄ carbon source in excess (in our case succinate), the anaplerotic sequence is not necessary, and is regulated independently of the operation of the taurine degradative pathway. The gene RSP_1980, annotated as malate synthase (*glcB*), is not transcribed under the tested conditions (Table 3), so it plays no role in the anaplerotic sequence; an alternative function for an orthologue in *Mycobacterium tuberculosis* has been demonstrated to be that of an adhesin (Kinhikar *et al.*, 2006).

During the dissimilation of taurine, not only the flow of carbon but also the fluxes of ammonia/ammonium ion and sulfite/sulfate ions must be contained. It seems logical to consider that the single copy of the *amtB* gene will be involved (e.g. Khademi *et al.*, 2004; Gorzynska *et al.*, 2006), but we obtained no reproducible data. The nature of the oxidation of sulfite to sulfate is also unknown. The sulfate is presumably excreted via the inducible TauZ (Fig. 1a, b, Table 3).

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REFERENCES

- Abraham, W.-R., Strömpl, C., Vancanneyt, M., Bennasar, A., Swings, J., Lünsdorf, H., Smit, J. & Moore, E. R. B. (2004). *Woodsholea maritima* gen. nov., sp. nov., a marine bacterium with a low diversity of polar lipids. *Int J Syst Evol Microbiol* **54**, 1227–1234.
- Alber, B. E., Spanheimer, R., Ebenau-Jehle, C. & Fuchs, G. (2006). Study of an alternative glyoxylate cycle for acetate assimilation by *Rhodobacter sphaeroides*. *Mol Microbiol* **61**, 297–309.
- Allen, J. A. & Garrett, M. R. (1971). Taurine in marine invertebrates. *Adv Mar Biol* **9**, 205–253.
- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Bergmeyer, H. U., Graßl, M. & Walter, E.-M. (1983). Phosphotransacetylase. In *Methods of Enzymatic Analysis*, pp. 295–296. Edited by H. U. Bergmeyer. Weinheim: Verlag Chemie.
- Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.
- Brüggemann, C., Denger, K., Cook, A. M. & Ruff, J. (2004). Enzymes and genes of taurine and isethionate dissimilation in *Paracoccus denitrificans*. *Microbiology* **150**, 805–816.
- Cook, A. M. (1987). Biodegradation of s-triazine xenobiotics. *FEMS Microbiol Rev* **46**, 93–116.
- Cook, A. M. & Hütter, R. (1981). s-Triazines as nitrogen sources for bacteria. *J Agric Food Chem* **29**, 1135–1143.
- Cook, A. M. & Denger, K. (2002). Dissimilation of the C₂ sulfonates. *Arch Microbiol* **179**, 1–6.
- Cook, A. M. & Denger, K. (2006). Metabolism of taurine in microorganisms: a primer in molecular diversity? *Adv Exp Med Biol* **583**, 3–13.
- Denger, K., Laue, H. & Cook, A. M. (1997). Anaerobic taurine oxidation: a novel reaction by a nitrate-reducing *Alcaligenes* sp. *Microbiology* **143**, 1919–1924.
- Denger, K., Ruff, J., Rein, U. & Cook, A. M. (2001). Sulfoacetaldehyde sulfo-lyase [EC 4.4.1.12] from *Desulfonisporea thiosulfatigenes*: purification, properties and primary sequence. *Biochem J* **357**, 581–586.
- Denger, K., Ruff, J., Schleheck, D. & Cook, A. M. (2004a). *Rhodococcus opacus* expresses the *xsc* gene to utilize taurine as a carbon source or as a nitrogen source but not as a sulfur source. *Microbiology* **150**, 1859–1867.
- Denger, K., Weinitschke, S., Hollemeyer, K. & Cook, A. M. (2004b). Sulfoacetate generated by *Rhodopseudomonas palustris* from taurine. *Arch Microbiol* **182**, 254–258.
- Denger, K., Smits, T. H. M. & Cook, A. M. (2006). L-Cysteate sulfo-lyase, a widespread, pyridoxal 5'-phosphate-coupled desulfonative enzyme purified from *Silicibacter pomeroyi* DSS-3^T. *Biochem J* **394**, 657–664.
- Desomer, J., Crespi, M. & Van Montagu, M. (1991). Illegitimate integration of non-replicative vectors in the genome of *Rhodococcus fascians* upon electrotransformation as an insertional mutagenesis system. *Mol Microbiol* **5**, 2115–2124.
- Dixon, G. H. & Kornberg, H. L. (1959). Assay methods for key enzymes of the glyoxylate cycle. *Biochem J* **72**, 3P.
- Eichhorn, E., van der Ploeg, J. R. & Leisinger, T. (2000). Deletion analysis of the *Escherichia coli* taurine and alkanesulfonate transport systems. *J Bacteriol* **182**, 2687–2795.
- Filatova, L. V., Berg, I. A., Krasil'nikova, E. N. & Ivanovsky, R. N. (2005). A study of the mechanism of acetate assimilation in purple nonsulfur bacteria lacking the glyoxylate shunt: enzymes of the citramalate cycle in *Rhodobacter sphaeroides*. *Microbiology* (English translation of *Mikrobiologiya*) **74**, 270–278.
- Forward, J. A., Behrendt, M. C., Wyborn, N. R., Cross, R. & Kelly, D. J. (1997). TRAP transporters: a new family of periplasmic solute transport systems encoded by the *dctPQM* genes of *Rhodobacter capsulatus* and by homologs in diverse gram-negative bacteria. *J Bacteriol* **179**, 5482–5493.
- Gesellschaft Deutscher Chemiker (1996). *German Standard Methods for the Laboratory Examination of Water, Waste Water and Sludge*. Weinheim: Verlag Chemie.
- Gorzynska, A. K., Denger, K., Cook, A. M. & Smits, T. H. M. (2006). Inducible transcription of genes involved in taurine uptake and dissimilation by *Silicibacter pomeroyi* DSS-3^T. *Arch Microbiol* **185**, 402–606.
- Hagen, K. D. & Nelson, D. C. (1997). Use of reduced sulfur compounds by *Beggiatoa* spp.: enzymology and physiology of marine and freshwater strains in homogeneous and gradient cultures. *Appl Environ Microbiol* **63**, 3957–3964.
- Huxtable, R. J. (1992). Physiological actions of taurine. *Physiol Rev* **72**, 101–163.
- Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (1990). *PCR Protocols. A Guide to Methods and Applications*. San Diego: Academic Press.
- Kappler, U. & Dahl, C. (2001). Enzymology and molecular biology of prokaryotic sulfite oxidation. *FEMS Microbiol Lett* **203**, 1–9.
- Kappler, U., Bennett, B., Rethmeier, J., Schwarz, G., Deutzmann, R., McEwan, A. G. & Dahl, C. (2000). Sulfite:cytochrome *c* oxidoreductase from *Thiobacillus novellus*. Purification, characterization, and molecular biology of a heterodimeric member of the sulfite oxidase family. *J Biol Chem* **275**, 13202–13212.
- Kertesz, M. A. (2000). Riding the sulfur cycle – metabolism of sulfonates and sulfate esters in Gram-negative bacteria. *FEMS Microbiol Rev* **24**, 135–175.
- Khademi, S., O'Connell, J., III, Remis, J., Robles-Colmenares, Y., Miercke, L. J. & Stroud, R. M. (2004). Mechanism of ammonia transport by Amt/MEP/Rh: structure of AmtB at 1.35 Å. *Science* **305**, 1587–1594.
- Kinhikar, A. G., Vargas, D., Li, H., Mahaffey, S. B., Hinds, L., Belisle, J. T. & Laal, S. (2006). *Mycobacterium tuberculosis* malate synthase is a laminin-binding adhesin. *Mol Microbiol* **60**, 999–1013.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Laue, H. & Cook, A. M. (2000a). Purification, properties and primary structure of alanine dehydrogenase involved in taurine metabolism in the anaerobe *Bilophila wadsworthia*. *Arch Microbiol* **174**, 162–167.
- Laue, H. & Cook, A. M. (2000b). Biochemical and molecular characterization of taurine:pyruvate aminotransferase from the anaerobe *Bilophila wadsworthia*. *Eur J Biochem* **267**, 6841–6848.
- Laue, H., Smits, T. H. M., Schumacher, U., Claros, M., Hartemink, R. & Cook, A. M. (2006). Identification of *Bilophila wadsworthia* in enrichment cultures by specific PCR which targets the taurine:pyruvate aminotransferase gene. *FEMS Microbiol Lett* **261**, 74–79.
- Lawrence, S. H., Luther, K. B., Schindelin, H. & Ferry, J. G. (2006). Structural and functional studies suggest a catalytic mechanism for the phosphotransacetylase from *Methanosarcina thermophila*. *J Bacteriol* **188**, 1143–1154.

- Lie, T. L., Leadbetter, J. R. & Leadbetter, E. R. (1998). Metabolism of sulfonic acids and other organosulfur compounds by sulfate-reducing bacteria. *Geomicrobiol J* **15**, 135–149.
- Masepohl, B., Führer, F. & Klipp, W. (2001). Genetic analysis of a *Rhodobacter capsulatus* gene region involved in utilization of taurine as a sulfur source. *FEMS Microbiol Lett* **205**, 105–111.
- Mayer, J., Denger, K., Smits, T. H. M., Hollemeyer, K., Groth, U. & Cook, A. M. (2006). *N*-Acetyltaurine dissimilated via taurine by *Delftia acidovorans* NAT. *Arch Microbiol* **186**, 61–67.
- Meister, M., Saum, S., Alber, B. E. & Fuchs, G. (2005). L-Malyl-coenzyme A/ β -methylmalyl-coenzyme A lyase is involved in acetate assimilation of the isocitrate lyase-negative bacterium *Rhodobacter capsulatus*. *J Bacteriol* **187**, 1415–1425.
- Novak, R. T., Gritzer, R. F., Leadbetter, E. R. & Godchaux, W. (2004). Phototrophic utilization of taurine by the purple nonsulfur bacteria *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*. *Microbiology* **150**, 1881–1891.
- Reichenbecher, W., Kelly, D. P. & Murrell, J. C. (1999). Desulfonation of propanesulfonic acid by *Comamonas acidovorans* strain P53: evidence for an alkanesulfonate sulfonase and an atypical sulfite dehydrogenase. *Arch Microbiol* **172**, 387–392.
- Rein, U., Gueta, R., Denger, K., Ruff, J., Hollemeyer, K. & Cook, A. M. (2005). Dissimilation of cysteate via 3-sulfolactate sulfo-lyase and a sulfate exporter in *Paracoccus pantotrophus* NKNCYSA. *Microbiology* **151**, 737–747.
- Ruff, J., Denger, K. & Cook, A. M. (2003). Sulphoacetaldehyde acetyltransferase yields acetyl phosphate: purification from *Alcaligenes defragrans* and gene clusters in taurine degradation. *Biochem J* **369**, 275–285.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor: Cold Spring Harbor Laboratory.
- Schiffer, A., Fritz, G., Kroneck, P. M. & Ermler, U. (2006). Reaction mechanism of the iron-sulfur flavoenzyme adenosine-5'-phosphosulfate reductase based on the structural characterization of different enzymatic states. *Biochemistry* **45**, 2960–2967.
- Sistrom, W. R. (1962). The kinetics of the synthesis of photopigments in *Rhodopseudomonas sphaeroides*. *J Gen Microbiol* **28**, 607–616.
- Sörbo, B. (1987). Sulfate: turbidimetric and nephelometric methods. *Methods Enzymol* **143**, 3–6.
- Styp von Rekowski, K., Denger, K. & Cook, A. M. (2005). Isethionate as a product from taurine during nitrogen-limited growth of *Klebsiella oxytoca* Tau-N1. *Arch Microbiol* **183**, 325–330.
- Thurnheer, T., Köhler, T., Cook, A. M. & Leisinger, T. (1986). Orphanic acid and analogues as carbon sources for bacteria: growth physiology and enzymic desulphonation. *J Gen Microbiol* **132**, 1215–1220.
- Vollrath, F., Fairbrother, W. J., Williams, R. J. P., Tillinghast, E. K., Bernstein, D. T., Gallagher, K. S. & Townley, M. A. (1990). Compounds in the droplets of the orb spider's viscid spiral. *Nature* **345**, 526–528.
- Weinitschke, S., Styp von Rekowski, K., Denger, K. & Cook, A. M. (2005). Sulfoacetaldehyde is excreted quantitatively by *Acinetobacter calcoaceticus* SW1 during growth with taurine as sole source of nitrogen. *Microbiology* **151**, 1285–1290.
- Weinitschke, S., Denger, K., Smits, T. H. M., Hollemeyer, K. & Cook, A. M. (2006). The sulfonated osmolyte *N*-methyltaurine is dissimilated by *Alcaligenes faecalis* and by *Paracoccus versutus* with release of methylamine. *Microbiology* **152**, 1179–1186.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Yin, M., Palmer, H. R., Fyfe-Johnson, A. L., Bedford, J. J., Smith, R. A. & Yancey, P. H. (2000). Hypotaurine, *N*-methyltaurine, taurine, and glycine betaine as dominant osmolytes of vestimentiferan tubeworms from hydrothermal vents and cold seeps. *Physiol Biochem Zool* **73**, 629–637.