A five-gene cluster involved in utilization of taurine-nitrogen and excretion of sulfoacetaldehyde by *Acinetobacter radioresistens* SH164

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Abstract *Acinetobacter calcoaceticus* SW1, under nitrogen limitation, assimilates the nitrogen moiety of taurine (2-aminoethanesulfonate) inducibly and excretes sulfoacetaldehyde, a product of taurine dehydrogenase (TauXY). BLAST searches of newly available genome sequences using the TauXY sequences revealed a 5-gene cluster, *tauRXYPI*, in *Acinetobacter radioresistens* SH164. We hypothesized that *tauXYPI* (HMPREF0018_00717–HMPREF0018_00720) encodes proteins that are orthologs of the undefined pathway from strain SW1, and that *tauR* (HMPREF0018_00716) encodes the relevant transcriptional regulator. Strain SH164 excreted sulfoacetaldehyde from taurine during growth. TauXY activity was expressed inducibly. Reverse transcription PCR showed that the *tauRXYPI* genes were transcribed inducibly. This allowed the conclusions that (i) TauP (currently annotated as permease GabP [TC 2.A.3]) is a taurine permease, and (ii) TauI (currently annotated as DUF6 drug/metabolite exporter [TC 2.A.7]) is a sulfoacetaldehyde exporter. The presumably equifunctional cluster *tauRXYPI* was then found in strain SW1. TauP is the third recognized taurine uptake system, and TauI is the third postulated class of sulfonate exporters, in bacteria.

Keywords *Acinetobacter* spp. · Assimilation of taurine-nitrogen · Excretion of sulfoacetaldehyde · Novel taurine permease · Novel sulfoacetaldehyde exporter

Abbreviations

RT-PCR	Reverse transcription PCR
TauABC	ABC transporter for taurine
tauI	Encodes sulfoacetaldehyde exporter
tauP	Encodes taurine permease
tauR	Encodes transcriptional regulator of taurine genes
tauXY	Encode taurine dehydrogenase

Introduction

Taurine (2-aminoethanesulfonate) is a major solute, and sometimes an osmolyte, in mammals, fish, red algae and marine creatures, and it is known as a carbon and energy source for terrestrial, freshwater and marine bacteria (e.g. Jones 1953; Allen and Garrett 1971; Kondo et al. 1971; Ito et al. 1977; Huxtable 1992; Yin et al. 2000; González et al. 2003; Rösch et al. 2008). Further, the compound is known as a sulfur source for the growth of bacteria (Eichhorn et al. 1997), and in this respect, TauR has been shown to be the transcriptional regulator (GntR-type) of taurine-sulfur metabolism in *Rhodobacter capsulatus* (Wiethaus et al.

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2008). Orthologs of *tauR* (>100 sequences in Genbank) are associated with most of the gene clusters known in bacteria for taurine degradation (Fig. S1) (e.g. Ruff et al. 2003; Denger et al. 2006a; Krejčík et al. 2008; Krejčík et al. 2010).

A recent addition to the metabolic reactions known for taurine is its utilization by bacteria as a nitrogen source, usually with concomitant excretion of an organosulfonate. The excretion of sulfoacetate (Denger et al. 2004b) was recently explained at the molecular level (Krejčík et al. 2008), as was the excretion of isethionate (2-hydroxyethanesulfonate) (Styp von Rekowski et al. 2005; Krejčík et al. 2010). The excretion of sulfoacetaldehyde, however, is known only as a physiological phenomenon in a representative organism, *Acinetobacter calcoaceticus* SW1 (Weinitschke et al. 2005). The deamination reaction in strain SW1 is catalyzed by taurine dehydrogenase, TauXY (*tauXY* is found in >51 genome sequences in Genbank), which has been explored elsewhere (Brüggemann et al. 2004; Weinitschke et al. 2007). Regulation, uptake of taurine and excretion of sulfoacetaldehyde (Fig. 1) remained undefined.

Analyses of newly available genome sequences indicated that a 5-gene cluster might encode the proteins of the unknown pathway (Fig. 1). This cluster includes the known genes *tauR* and *tauXY*, and the genes *tauP* (*gabP*-like; amino acid/polyamine/organocation (APC) superfamily [TC 2.A.3], Niegemann et al. 1993) (Fig. 1) as a candidate taurine permease (see 'Discussion') and *tauI* as a candidate sulfoacetaldehyde exporter (DUF6, drug/metabolite exporter (DME) [TC 2.A.7.3.-]) (Jack et al. 2001). The gene cluster was found in the genomes of *A. radioresistens* strains SK82 and SH164. The latter organism was readily available, which allowed us to explore the hypothesis.

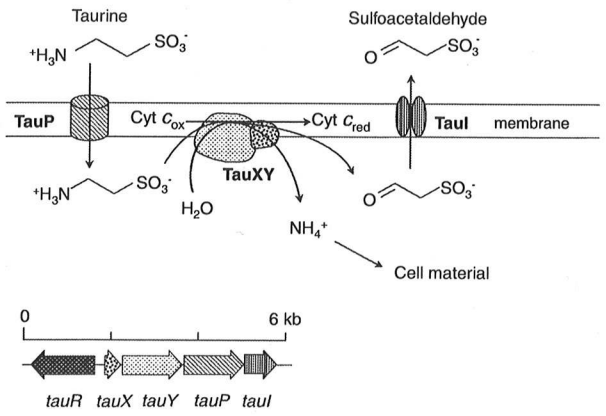

Fig. 1 Illustration of the published pathway of taurine-nitrogen utilization and sulfoacetaldehyde formation and excretion (Weinitschke et al. 2005) adapted to the candidate genes of *A. radioresistens* SH164. The map shows the cluster of candidate genes (HMPREF0018_00716–HMPREF0018_00720; *tauR–tauI*)

Methods and materials

Materials

Taurine (≥99.0 %) was purchased from Fluka. Sulfoacetaldehyde, as the bisulfite addition complex, was synthesized and characterized previously (Denger et al. 2001). Other chemicals were of the highest purity available and were purchased from Biomol, Fluka, Merck, Roth, Serva or Sigma. Glutamate dehydrogenase [EC 1.4.1.3] was from Sigma.

Organisms, growth media, growth conditions and cell extracts

Acinetobacter radioresistens SH164 (CCUG 57822) was purchased from the Culture Collection of the University of Göteborg (Sweden). *A. calcoaceticus* SW1 (DSM16962) was isolated in this laboratory (Weinitschke et al. 2005). Each organism was routinely grown at 30 °C in the dark in potassium phosphate-buffered medium supplied with 10 mM acetate-carbon and 2 mM taurine-nitrogen or 2 mM ammonium chloride as described previously (Weinitschke et al. 2005).

Precultures (5 ml) of strain SH164 were grown in 30-ml screw-cap tubes in a roller. Growth experiments were done on a 50-ml scale in shaken 300-ml Erlenmeyer flasks. Samples were taken at intervals to measure optical density (at 580 nm; OD_{580}), to assay protein, and to determine the concentrations of taurine, sulfoacetaldehyde and the ammonium and sulfate ions. Similar cultures were used to generate cells for enzyme assays and molecular analyses. Harvesting and cell disruption with a French pressure cell were done as described elsewhere (Denger et al. 2004a).

Cells for the preparation of total RNA were harvested in the early exponential phase of growth (OD_{580} 0.14–0.16) by centrifugation at 5,000*g*. Suspended cells were stored in RNA*later* stabilizing solution (Ambion/Applied Biosystems, Austin, TX, USA), as recommended by the manufacturer, prior to extraction of RNA.

Analytical methods and enzyme assay

Growth was followed turbidimetrically as OD_{580} (for *A. radioresistens* SH164 $OD_{580} = 1 = 269$ mg protein l^{-1}) and quantified as protein in whole cells in a Lowry-type reaction (Cook and Hütter 1981). Protein in cell extracts was assayed by protein-dye binding (Bradford 1976). Bovine serum albumin was used as a standard in each case. Taurine was determined by HPLC after derivatization with 2,4-dinitrofluorobenzene (Denger et al. 1997). Sulfoacetaldehyde was derivatized with 2-(diphenylacetyl)indane-1,3-dione-1-hydrazone and assayed by HPLC (Cunningham

et al. 1998); the compound was determined by its reaction with the highly specific sulfoacetaldehyde reductase (EC 1.1.1.313) (Krejčík et al. 2010), and its identity was confirmed by MALDI-TOF-MS used in the negative ion mode (Weinitschke et al. 2005). Sulfoacetate and isethionate were quantified by ion chromatography (Denger et al. 2004b; Styp von Rekowski et al. 2005). The ammonium ion was determined enzymically by its reaction with glutamate dehydrogenase (Bergmeyer and Beutler 1984). Sulfate was measured as the optical density of a suspension of insoluble $BaSO_4$ (Sörbo 1987). Taurine dehydrogenase (TauXY) was assayed as the reduction of 2,6-dichlorophenolindophenol (Brüggemann et al. 2004).

Molecular methods

Primers (Table S1) for PCR and RT-PCR were synthesized by Microsynth (Balgach, Switzerland). Total RNA was isolated using the E.Z.N.A. bacterial RNA kit (Omega Bio-Tek, Doraville, USA) or the peqGOLD TriFast DNA/RNA/protein purification system (PEQLAB Ltd., Fareham, UK) following the manufacturers' instructions. Isolated RNA was tested for residual DNA by PCR using the primer pair 16S-27f–16S-533r (Table S1); contaminant DNA was removed with RNase-free DNase I (50 units/µl) (Fermentas, St. Leon-Rot, Germany) following the manufacturer's instruction. M-MuLV reverse transcriptase (20 units/µl) was from Fermentas, and the RT reactions were set up as recommended by the supplier using the reverse PCR primers listed in Table S1. cDNA integrity after RT was tested by PCR using the 16S rRNA-specific primers 16S-27f and 16S-533r (Weisburg et al. 1991). Chromosomal DNA of strain SH164 was used as a positive control for PCRs. *Taq* DNA Polymerase (5 units/µl) was from Fermentas, and the PCR mixture (routinely 25 µl) contained: 1× Taq buffer with $(NH_4)_2SO_4$, 10 % DMSO, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs (each), 0.4 µM forward primer, 0.4 µM reverse primer, 0.5 µg template DNA, 2.5 units of *Taq* DNA polymerase and water (up to 25 µl of total volume). Cycling conditions were as follows: initial denaturation for 3 min at 95 °C, and for 35 cycles denaturation for 0.5 min at 95 °C, primer annealing for 0.5 min at 49 °C (for 16S-27f and 16S-533r primers) or at 58 °C (for all the other primers), extension for either 0.5 min or longer (calculated as 1 min per 1 kb of PCR fragment) at 72 °C, and final extension for 5 min at 72 °C. PCR products were visualized on 1.5 or 2 % agarose gels stained with ethidium bromide (Sambrook et al. 1989); a 50-bp DNA ladder (Fermentas) was used as a marker.

Chromosomal DNA was isolated from bacteria as described elsewhere (Desomer et al. 1991). PCR products for sequencing were amplified using Phusion High-Fidelity DNA Polymerase (Finnzymes) (with proofreading activity), purified using the E.Z.N.A. Cycle-pure Kit (Omega Bio-Tek) and submitted for sequencing (GATC Biotech, Konstanz, Germany) using appropriate primers as listed in Table S1.

Software for sequence analyses and accession numbers

Analyses of the genome sequences of *A. radioresistens* SH164 (accession no. NZ_ACPO00000000), *A. radioresistens* SK82 (accession no. NZ_ACVR00000000) and *Acinetobacter* sp. strain SH024 (accession no. NZ_ADCH00000000) were done using the BLASTP algorithm on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) and the IMG Data Management & Analysis Systems (http://img.jgi.doe.gov). Signal peptide prediction was done using the SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP) (Bendtsen et al. 2004). Primers for RT and PCR were designed using the program PrimerSelect (version 5.07) subroutine of LASERGENE program package (DNASTAR, Madison, USA). Phylogenetic trees were constructed from amino acid sequence alignments done with ClustalX (http://www.clustal.org) and visualized using Dendroscope (http://ab.inf.uni-tuebingen.de).

Results

Growth of *A. radioresistens* and the formation of sulfoacetaldehyde by TauXY

Acinetobacter radioresistens SH164 grew in acetate-salts medium with the ammonium ion as the source of nitrogen. The molar growth yield in acetate/ammonium salts medium was 49 ± 2 g protein $(\text{mol N})^{-1}$, consistent with complete utilization of the ammonium ion (Cook 1987), which disappeared during growth. There was no growth in the absence of a source of combined nitrogen. Strain SH164 did not grow in taurine-salts medium with (or without) the ammonium ion, so the organism did not utilize the taurine-carbon. However, the organism did grow in acetate-salts medium with taurine as the sole source of nitrogen. The molar growth yield in acetate-/taurine-salts medium was 53 ± 2 g protein $(\text{mol N})^{-1}$ (Fig. 2), representing quantitative utilization of taurine-nitrogen; no transient excretion of ammonium ion was detected in the growth medium (Fig. 2). Further, no sulfate was released into the medium (Fig. 2).

Taurine disappeared concomitantly with growth in acetate-/taurine-salts medium (Fig. 2), and in its place, we found a compound which, after derivatization, co-chromatographed with derivatized authentic sulfoacetaldehyde (data not shown). Furthermore, the compound was a substrate for the highly specific sulfoacetaldehyde reductase

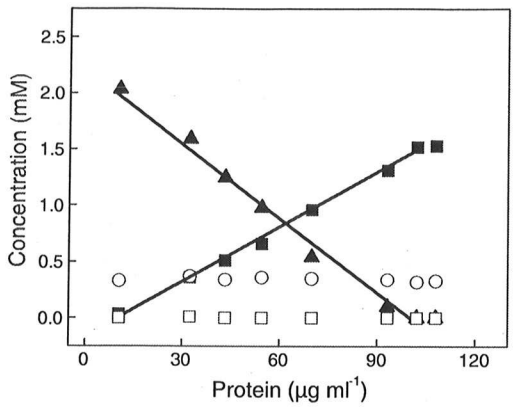

Fig. 2 Linearized growth curve of *A. radioresistens* SH164 with taurine as the sole nitrogen source in acetate-salts medium. Concentrations of substrate and product are shown as a function of growth (as protein concentration). Key: *filled triangles* taurine, *filled squares* sulfoacetaldehyde, *open circles* sulfate ion, *open squares* ammonium ion

(Fig. 2). These tentative identifications of sulfoacetaldehyde were confirmed by MALDI-TOF-MS in the negative ion mode. There was a novel signal at $m/z = 123 = [M - 1]^-$ in outgrown medium, identical with that of authentic sulfoacetaldehyde [$M = 124$] (not shown). Whereas sulfoacetaldehyde was excreted concomitantly with growth and thoroughly identified, its quantification varied from about 75 % (Fig. 2) to >90 % (preliminary experiments) of the expected yield. Neither isethionate nor sulfoacetate (Krejčík et al. 2008; Krejčík et al. 2010) was formed, so the fate of some of the taurine-carbon may be unknown. Nonetheless, a pathway to sulfoacetaldehyde exists (Fig. 2), and we explored it.

The pathway to sulfoacetaldehyde was presumed to involve only one scalar enzyme, TauXY (Fig. 1). There was no detectable TauXY in acetate-/ammonium-grown cells. In contrast, membrane-associated TauXY (0.3 mkat (kg protein)$^{-1}$) was detected in extracts of acetate/taurine-grown cells of *A. radioresistens*. TauXY was obviously inducible.

Inducible genes identified by RT-PCR in *A. radioresistens*

The inducibility of TauXY and the observed excretion of sulfoacetaldehyde supported the hypothesis that the genes surrounding the single copy of *tauXY* on the genome would encode uptake of taurine and excretion of sulfoacetaldehyde (Fig. 1). RT-PCR data showed that there was no detectable transcription of *tauXYPI* during growth in acetate/ammonium salts medium (Fig. 3). In contrast, acetate/taurine-grown cells showed transcription of *tauXYPI* (Fig. 3). Gene *tauR* was also subject to inducible transcription (Fig. 3).

The *tauRXYPI* cluster in *A. calcoaceticus* SW1

Orthologs of the *tauRXYPI* gene cluster in *A. radioresistens* SH164 are present in the genomes of *A. radioresistens* SK82 and *Acinetobacter* sp. strain SH024. The locus tags are ACIRA0001_2517–ACIRA0001_2521 and HMPREF0013_03350–HMPREF0013_03354, respectively. We hypothesized that this 6-kbp gene cluster, *tauRXYPI* from *A. radioresistens* SH164, would have an ortholog in the first organism found to excrete sulfoacetaldehyde, *A. calcoaceticus* SW1. A 5-kbp amplicon was obtained from the strain SW1 (data not shown) in the reaction with the primer pair *AR_tauR_F* and AR_tauP_R (see Table S1) and sequenced completely using the primers listed in Table S1. The sequence analysis of this 5-kbp fragment confirmed the presence of a *tauRXYP* cluster (cf. Fig. 1) in *A. calcoaceticus* SW1. Furthermore, downstream of *tauP* in strain SW1, a

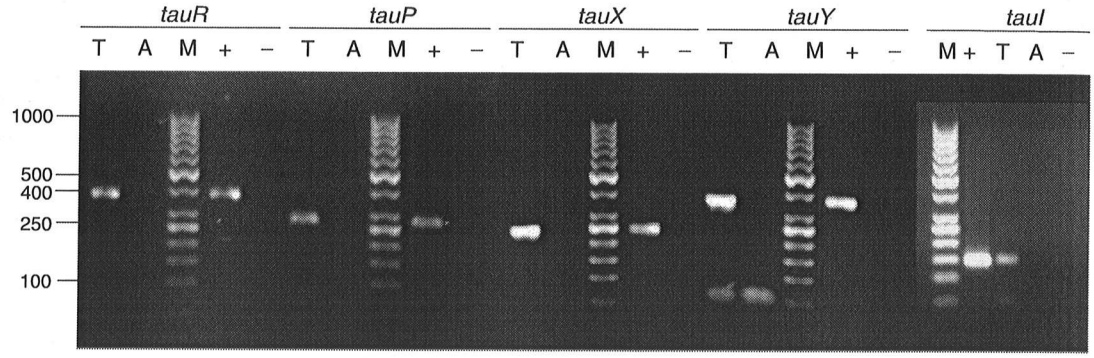

Fig. 3 Transcriptional analysis of the *tauRXYPI* gene cluster in *A. radioresistens* SH164. The primer pairs used for reverse transcription (RT) of mRNA and for subsequent PCR with the cDNA as template are indicated in Table S1. Labeling of lanes: PCR with cDNA as template derived by RT of mRNA prepared from cells grown in acetate/taurine (T) and acetate/ammonium (A) salts medium; (M), length marker with relevant sizes indicated at the left-hand side of the figure; (+), PCR positive control with chromosomal DNA as template; (−), control reactions for DNA contamination with total RNA from acetate-/taurine-grown cells. Lengths of shown PCR fragments from *tauR* to *tauI* are 372, 272, 238, 348 and 146 bp, respectively

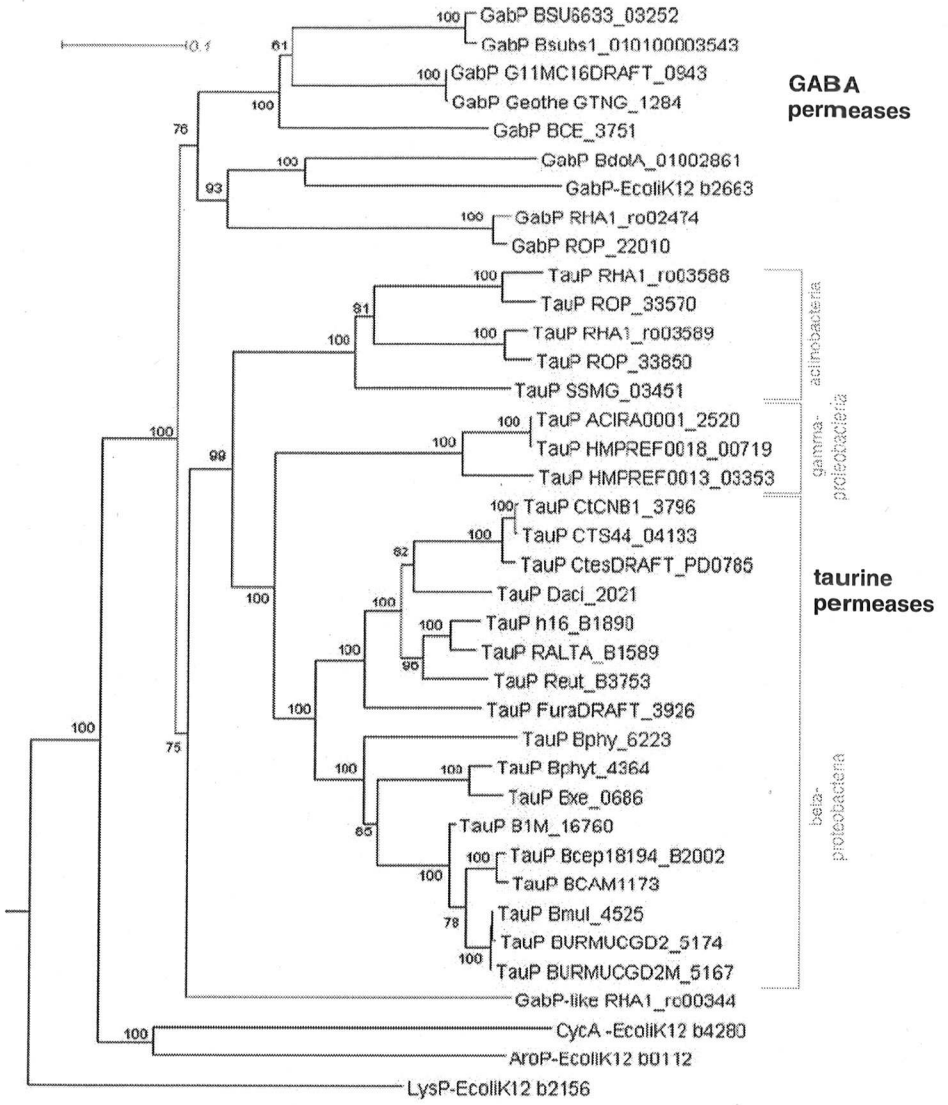

Fig. 4 Dendrogram illustrating the sequence divergence of TauP and GabP proteins within the amino acid permease (AAT) family (TC 2.A.3.1) of the amino acid/polyamine/ organocation (APC) superfamily (TC 2.A.3.-) of transporters. The neighbor-joining dendrogram shows the estimated relationship of TauP and GabP indicated by a ClustalX sequence alignment. The proteins used are identified by their appropriate locus tag. The building of the tree involved bootstrapping (100 times). The *bar* represents 10 % sequence difference. Sequences of typical AAT-family amino acid permeases (CycA, AroP and LysP of *Escherichia coli* K12) were included in the alignment, and an amino acid permease of *Saccharomyces* (not shown; GAP1 general amino acid permease, locus tag YKR039W) was used to root the tree

0.5-kbp fragment of a *tauI* gene was amplified (using primer pair AR_tauP_F and AC_R4 [Table S1]) and sequenced. The 5.5-kbp fragments in each organism share similarity of >92% on nucleotide level. Hence, the pathway proteins in *A. calcoaceticus* SW1 are presumably encoded by this ortholog of the *tauRXYPI* cluster in *A. radioresistens* SH164.

Discussion

The presence of the gene cluster, *tauRXYRI*, in *A. radioresistens* SH164 allowed one aspect of the physiology of the organism to be predicted, namely utilization of taurine-nitrogen via TauXY and excretion of sulfoacetaldehyde. Correspondingly, the same physiology in *A. calcoaceticus* SW1 allowed the prediction of a similar *tauRXYRI* cluster on its genome.

The activity of TauXY, and the confirmation by RT-PCR that the *tauXY* genes were transcribed inducibly (Fig. 3), showed that *tauXY* were translated. So it is reasonable to assume that the *tauPI* genes are not only transcribed (Fig. 3), but also translated. TauR as regulator for taurine-degradative genes is found in alpha-, beta- and gammaproteobacteria as well as in actinobacteria (Fig. S1), and in *A. radioresistens* SH164. This GntR-type transcriptional regulator (Wiethaus et al. 2008) is apparently subject to autoregulation (Fig. 3) as reported for another GntR-type regulator (Belitsky and Sonenshein 2002).

The sulfonate group makes organosulfonates impermeable to bacterial cell membranes unless a transporter is

present (Graham et al. 2002; Mampel et al. 2004; Weinitschke et al. 2010). TauP, with 12 predicted transmembrane helices, and TauI, with 10 predicted transmembrane helices, were presumed to be involved in membrane transport. Orthologs of *tauP* (annotated *gabP*, GABA permease (γ- or) 4-aminobutyrate permease (e.g. Niegemann et al. 1993)), adjacent to clusters of taurine-relevant genes, have been observed since our first access to genome sequences (Ruff et al. 2003). Their meaning was unclear, because the established ABC-type transporter, TauABC (Eichhorn et al. 2000), was usually present (e.g. Ruff et al. 2003); alternatively, a TRAP-type transporter, TauKLM, was found (e.g. Brüggemann et al. 2004; Denger et al. 2006a). It was only when the genome sequence of *Delftia acidovorans* SPH-1 (NC_010002) became available in which neither *tauABC* nor *tauKLM* occurred, that TauP was recognized as a probable taurine permease. A dendrogram of an alignment of relevant TauP and GapP sequences shows that they clustered each into separate, deeply branched clades within the group of amino acid permease family transporters (AA_permease; TC 2.A.3.1, PF00324) (Fig. 4). We thus conclude that there are now three known taurine transporters, TauABC, TauKLM and novel TauP.

Up till now, two types of sulfonate exporters are known, the TauE-type (annotated 'DUF81') and the TauZ-type (annotated 'conserved protein 689'); some organisms contain neither, so other exporters are anticipated. TauE was initially presumed to be a sulfate exporter, until the sulfite dehydrogenases involved were confirmed to be periplasmic enzymes, as known elsewhere (Kappler et al. 2000; Ruff et al. 2003; Weinitschke et al. 2007; Denger et al. 2008; Wilson and Kappler 2009). TauE was then thought to be specific for sulfite, but more recent data indicate that organosulfonates are also exported by TauE (Weinitschke et al. 2007; Krejčík et al. 2008; Krejčík et al. 2010), sometimes with a broad substrate range (Mayer and Cook 2009). The TauZ-type of transporters (and orthologs, e.g. CuyZ Denger et al. 2006b), initially also thought to be sulfate exporters (Brüggemann et al. 2004), are now presumed to be sulfite exporters (Denger et al. 2009). The inducible transcription of *tauI* (Fig. 3) from *A. radioresistens* SH164 during growth with taurine-nitrogen, and the identical sequence in strain SK82 and the orthologous *tauRXYPI* in *Acinetobacter* sp. strain SH024 and in *A. calcoaceticus* SW1, implies the inducible expression of TauI. Furthermore, its annotation as 'drug/metabolite exporter' family protein [TC 2.A.7.3.-] (Jack et al. 2001), in the present connotation, allows its tentative identification as a sulfoacetaldehyde exporter. We thus conclude that there are at least three types of sulfite/sulfonate exporters, TauE, TauZ and novel TauI.

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References

Allen JA, Garrett MR (1971) Taurine in marine invertebrates. Adv Mar Biol 9:205–253

Belitsky BR, Sonenshein AL (2002) GabR, a member of a novel protein family, regulates the utilization of gamma-aminobutyrate in *Bacillus subtilis*. Mol Microbiol 45:569–583

Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004) Improved prediction of signal peptides: SignalP 3.0. J Mol Biol 340: 783–795

Bergmeyer HU, Beutler H-O (1984) Ammonia. In: Bergmeyer HU (ed) Methods of enzymatic analysis, 3rd edn. Verlag Chemie, Weinheim, pp 454–461

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 AM, Ruff J (2004) Enzymes and genes of taurine and isethionate dissimilation in *Paracoccus denitrificans*. Microbiology (Reading, UK) 150:805–816

Cook AM (1987) Biodegradation of *s*-triazine xenobiotics. FEMS Microbiol Rev 46:93–116

Cook AM, Hütter R (1981) *s*-Triazines as nitrogen sources for bacteria. J Agric Food Chem 29:1135–1143

Cunningham C, Tipton KF, Dixon HBF (1998) Conversion of taurine into *N*-chlorotaurine (taurine chloramine) and sulphoacetaldehyde in response to oxidative stress. Biochem J 330:939–945

Denger K, Laue H, Cook AM (1997) Anaerobic taurine oxidation: a novel reaction by a nitrate-reducing *Alcaligenes* sp. Microbiology (Reading, UK) 143:1919–1924

Denger K, Ruff J, Rein U, Cook AM (2001) Sulfoacetaldehyde sulfolyase [EC 4.4.1.12] from *Desulfonispora thiosulfatigenes*: purification, properties and primary sequence. Biochem J 357:581–586

Denger K, Ruff J, Schleheck D, Cook AM (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 (Reading, UK) 150:1859–1867

Denger K, Weinitschke S, Hollemeyer K, Cook AM (2004b) Sulfoacetate generated by *Rhodopseudomonas palustris* from taurine. Arch Microbiol 182:254–258

Denger K, Smits THM, Cook AM (2006a) Genome-enabled analysis of the utilization of taurine as sole source of carbon or nitrogen by *Rhodobacter sphaeroides* 2.4.1. Microbiology (Reading, UK) 152:3167–3174

Denger K, Smits THM, Cook AM (2006b) L-Cysteate sulfo-lyase, a widespread, pyridoxal 5′-phosphate-coupled desulfonative enzyme purified from *Silicibacter pomeroyi* DSS-3^T. Biochem J 394:657–664

Denger K, Weinitschke S, Smits THM, Schleheck D, Cook AM (2008) Bacterial sulfite dehydrogenases in organotrophic metabolism: separation and identification in *Cupriavidus necator* H16 and in *Delftia acidovorans* SPH-1. Microbiology (Reading, UK) 154:256–263

Denger K, Mayer J, Buhmann M, Weinitschke S, Smits THM, Cook AM (2009) Bifurcated degradative pathway of 3-sulfolactate in *Roseovarius nubinhibens* ISM via sulfoacetaldehyde acetyltransferase and (*S*)-cysteate sulfo-lyase. J Bacteriol 191:5648–5656

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

Eichhorn E, van der Ploeg JR, Kertesz MA, Leisinger T (1997) Characterization of α-ketoglutarate-dependent taurine dioxygenase from *Escherichia coli*. J Biol Chem 272:23031–23036

Eichhorn E, van der Ploeg JR, Leisinger T (2000) Deletion analysis of the *Escherichia coli* taurine and alkanesulfonate transport systems. J Bacteriol 182:2687–2795

González JM et al (2003) *Silicibacter pomeroyi* sp. nov. and *Roseovarius nubinhibens* sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. Int J Syst Evol Microbiol 53:1261–1269

Graham DE, Xu H, White RH (2002) Identification of coenzyme M biosynthetic phosphosulfolactate synthase: a new family of sulfonate biosynthesizing enzymes. J Biol Chem 277:13421–13429

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72:101–163

Ito K, Miyazawa K, Matsumoto F (1977) Amino acid composition of the ethanolic extractives from 31 species of marine red algae. Hiroshima Daigaku Suichikusangakubu Kiyo 16:77–90

Jack DL, Yang NM, Saier MH Jr (2001) The drug/metabolite transporter superfamily. Eur J Biochem 268:3620–3639

Jones NR (1953) Taurine in fresh and iced skeletal muscle of codling (*Gadus callarias*). Biochem J 56:xxii

Kappler U, Bennett B, Rethmeier J, Schwarz G, Deutzmann R, McEwan AG, 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

Kondo H, Anada H, Ohsawa K, Ishimoto M (1971) Formation of sulfoacetaldehyde from taurine in bacterial extracts. J Biochem 69:621–623

Krejčík Z, Denger K, Weinitschke S, Hollemeyer K, Pačes V, Cook AM, Smits THM (2008) Sulfoacetate released during the assimilation of taurine-nitrogen by *Neptuniibacter caesariensis*: purification of sulfoacetaldehyde dehydrogenase. Arch Microbiol 190:159–168

Krejčík Z, Hollemeyer K, Smits THM, Cook AM (2010) Isethionate formation from taurine in *Chromohalobacter salexigens*: purification of sulfoacetaldehyde reductase. Microbiology (Reading, UK) 156:1547–1555

Mampel J, Maier E, Tralau T, Ruff J, Benz R, Cook AM (2004) A novel outer-membrane anion channel (porin) as part of the putatively two-component transport system for *p*-toluenesulfonate in *Comamonas testosteroni* T-2. Biochem J 383:91–99

Mayer J, Cook AM (2009) Homotaurine metabolized to 3-sulfopropanoate in *Cupriavidus necator* H16: enzymes and genes in a patchwork pathway. J Bacteriol 191:6052–6058

Niegemann E, Schulz A, Bartsch K (1993) Molecular organization of the *Escherichia coli gab* cluster: nucleotide sequence of the structural genes *gabD* and *gabP* and expression of the GABA permease gene. Arch Microbiol 160:454–460

Rösch V, Denger K, Schleheck D, Smits THM, Cook AM (2008) Different bacterial strategies to degrade taurocholate. Arch Microbiol 190:11–18

Ruff J, Denger K, Cook AM (2003) Sulphoacetaldehyde acetyltransferase yields acetyl phosphate: purification from *Alcaligenes defragrans* and gene clusters in taurine degradation. Biochem J 369:275–285

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbour

Sörbo B (1987) Sulfate: turbidimetric and nephelometric methods. Methods Enzymol 143:3–6

Styp von Rekowski K, Denger K, Cook AM (2005) Isethionate as a product from taurine during nitrogen-limited growth of *Klebsiella oxytoca* Tau-N1. Arch Microbiol 183:325–330

Weinitschke S, Styp von Rekowski K, Denger K, Cook AM (2005) Sulfoacetaldehyde is excreted quantitatively by *Acinetobacter calcoaceticus* SW1 during growth with taurine as sole source of nitrogen. Microbiology (Reading, UK) 151:1285–1290

Weinitschke S, Denger K, Cook AM, Smits THM (2007) The DUF81 protein TauE in *Cupriavidus necator* H16, a sulfite exporter in the metabolism of C_2-sulfonates. Microbiology (Reading, UK) 153:3055–3060

Weinitschke S, Hollemeyer K, Kusian B, Bowien B, Smits THM, Cook AM (2010) Sulfoacetate is degraded via a novel pathway involving sulfoacetyl-CoA and sulfoacetaldehyde in *Cupriavidus necator* H16. J Biol Chem 285:35249–35254

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

Wiethaus J, Schubert B, Pfander Y, Narberhaus F, Masepohl B (2008) The GntR-like regulator TauR activates expression of taurine utilization genes in *Rhodobacter capsulatus*. J Bacteriol 190:487–493

Wilson JJ, Kappler U (2009) Sulfite oxidation in *Sinorhizobium meliloti*. Biochim Biophys Acta 1787:1516–1525

Yin M, Palmer HR, Fyfe-Johnson AL, Bedford JJ, Smith RA, Yancey PH (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