

The sulfonated osmolyte *N*-methyltaurine is dissimilated by *Alcaligenes faecalis* and by *Paracoccus versutus* with release of methylamine

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Selective enrichments yielded bacterial cultures able to utilize the osmolyte *N*-methyltaurine as sole source of carbon and energy or as sole source of fixed nitrogen for aerobic growth. Strain MT1, which degraded *N*-methyltaurine as a sole source of carbon concomitantly with growth, was identified as a strain of *Alcaligenes faecalis*. Stoichiometric amounts of methylamine, whose identity was confirmed by matrix-assisted, laser-desorption ionization time-of-flight mass spectrometry, and of sulfate were released during growth. Inducible *N*-methyltaurine dehydrogenase, sulfoacetaldehyde acetyltransferase (Xsc) and a sulfite dehydrogenase could be detected. Taurine dehydrogenase was also present and it was hypothesized that taurine dehydrogenase has a substrate range that includes *N*-methyltaurine. Partial sequences of a *tauY*-like gene (encoding the putative large component of taurine dehydrogenase) and an *xsc* gene were obtained by PCR with degenerate primers. Strain N-MT utilized *N*-methyltaurine as a sole source of fixed nitrogen for growth and could also utilize the compound as sole source of carbon. This bacterium was identified as a strain of *Paracoccus versutus*. This organism also expressed inducible (*N*-methyl)taurine dehydrogenase, Xsc and a sulfite dehydrogenase. The presence of a gene cluster with high identity to a larger cluster from *Paracoccus pantotrophus* NKNCYSA, which is now known to dissimilate *N*-methyltaurine via Xsc, allowed most of the overall pathway, including transport and excretion, to be defined. *N*-Methyltaurine is thus another compound whose catabolism is channelled directly through sulfoacetaldehyde.

INTRODUCTION

Taurine (2-aminoethanesulfonate) is widespread in the biosphere, whether as the free ampholyte or in derivatized form. Mammals usually contain about 8 mM taurine, which they cannot desulfonate (Huxtable, 1992), and which they excrete largely in urine (e.g. Stipanuk, 2004). We presume that this steady supply of taurine to the environment explains the considerable diversity of microbial taurine metabolism, which includes utilizing the compound as a sole source of carbon, nitrogen or sulfur for aerobic growth, as a source of,

or as a sink for, electrons for anaerobes, or as a fermentative substrate (Cook & Denger, 2006).

One of the few undisputed functions of free taurine in many animals is its role as an osmolyte (Huxtable, 1992; Yancey *et al.*, 2002). One widespread derivative of taurine, *N*-methyltaurine (Allen & Garrett, 1971), has now been recognized as an osmolyte in some deep-sea worms, where it can be present at concentrations in excess of 250 mM (Yin *et al.*, 2000). The fate of this compound was unknown until now.

Abbreviations: DCPIP, dichlorophenol indophenol; MALDI-TOF-MS, matrix-assisted laser-desorption ionization time-of-flight mass spectrometry.

The GenBank/EMBL/DBJ accession numbers for the partial sequences in *Alcaligenes faecalis* MT1 reported in this paper are: 16S rRNA gene, AM048879; putative large subunit of taurine dehydrogenase (*tauY*), AM048880; sulfoacetaldehyde acetyltransferase (*xsc*), AM048881. The sequences for *Paracoccus versutus* N-MT are: 16S rRNA gene, AM048882; *tauCXY-xsc* gene cluster, AM087667; *tauZ-pta* gene cluster, AM087668.

The bacterial catabolism of taurine and of the structurally related compounds isethionate (2-hydroxyethanesulfonate), sulfoacetate and ethane-1,2-disulfonate converges at sulfoacetaldehyde (Cook & Denger, 2002). We were interested to see whether *N*-methyltaurine would be degradable, and if so, whether sulfoacetaldehyde acetyltransferase (Xsc) (EC 2.3.3.15) was involved as well (Fig. 1). Xsc catalyses the phosphatolysis of sulfoacetaldehyde to sulfite and acetyl phosphate, and three subgroups of *xsc* genes have been

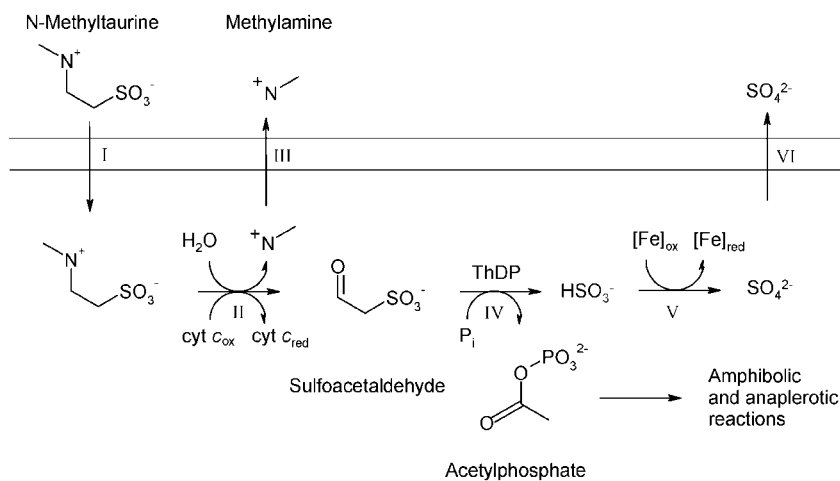


Fig. 1. Degradative pathway for *N*-methyltaurine proposed for *A. faecalis* MT1 and the transport functions and ancillary reactions also required. We hypothesize an ABC transporter for *N*-methyltaurine (I) (see text). An inducible *N*-methyltaurine dehydrogenase (II) was discovered in this study. Inducible sulfoacetaldehyde acetyltransferase (IV) and a ferricyanide-dependent sulfite dehydrogenase (V) were detected. The export of methylamine (III) and sulfate (VI) is deduced from earlier work (e.g. Brüggemann *et al.*, 2004; Rein *et al.*, 2005).

detected in different phylogenetic groups (Ruff *et al.*, 2003; Brüggemann *et al.*, 2004).

Further hypotheses on the dissimilation of *N*-methyltaurine involved the nature of removal of the methyl group, possibly by its oxygenation to yield taurine, followed by either taurine: pyruvate aminotransferase (EC 2.6.1.77) with alanine dehydrogenase (Laue & Cook, 2000; Denger *et al.*, 2004a) to yield sulfoacetaldehyde, or by taurine:ferricytochrome-*c* oxidoreductase (deaminating) (taurine dehydrogenase, presumably EC 1.4.2.–) to yield sulfoacetaldehyde (Brüggemann *et al.*, 2004). Alternatively, the reaction might proceed by hydrolytic removal of the methylamino moiety to yield sulfoacetaldehyde, analogous to reactions (e.g. AtzB and AtzC) observed in the degradation of *N*-alkylated triazines (Cook, 1987; Martinez *et al.*, 2001).

In addition to metabolic enzymes in the pathway, a range of membrane-related phenomena must also be considered during the dissimilation of organosulfonates. The outer and inner cell membranes of Gram-negative cells are impermeable to sulfonates (Graham *et al.*, 2002; Mampel *et al.*, 2004) and the transport of taurine into the cell has been associated with ATP-binding cassette (ABC) or tripartite ATP-independent periplasmic (TRAP) transport systems (Kertesz, 2000; Brüggemann *et al.*, 2004), so transport of *N*-methyltaurine (I in Fig. 1) may be presumed to be energy dependent. A range of excretion proteins is also postulated (Fig. 1). There are candidate genes to encode these functions in several organisms. In a range of α -Proteobacteria, e.g. *Paracoccus pantotrophus* NKNCYSA, a regulated eight-gene cluster, *tauABCXY-xsc-tauZ-pta*, is believed to encode an ABC transporter for taurine (TauABC), taurine dehydrogenase (TauXY), Xsc, a putative sulfate exporter (TauZ) and phosphotransacetylase (Pta) (Brüggemann *et al.*, 2004; Rein *et al.*, 2005; J. Ruff & T. H. M. Smits, unpublished). In other organisms, e.g. *Paracoccus denitrificans* NKNIS, a TRAP taurine transporter is predicted in an analogous, regulated, eight-gene cluster (Brüggemann *et al.*, 2004): in a sequencing project, there is a single taurine cluster on the partially sequenced genome of *P. denitrificans* PD1222 ([genome.ornl.gov/microbial/pden/\). Several \$\beta\$ -Proteobacteria seem to have a similar pathway with an ABC transporter, but the genes are interspersed in small units over 15–30 kbp \(Brüggemann *et al.*, 2004\). There is direct evidence, transcription of the genes, for the involvement of TauABC in the dissimilation of taurine in *Silicibacter pomeroyi* DSS-3 \(A. K. Gorzynska, K. Denger, A. M. Cook & T. H. M. Smits, unpublished data\). The overall degradative pathway \(Fig. 1\) also requires a sulfite dehydrogenase \(EC 1.8.2.1\), which seems to be regulated independently of taurine metabolism, apparently responding to sulfite production \(Brüggemann *et al.*, 2004\). There would appear to be at least two types of sulfite dehydrogenase, those coupled to cytochrome *c* \(SorAB; Kappler *et al.*, 2000\), and those which can be assayed in the presence of ferricyanide \(Reichenbecher *et al.*, 1999\) and for which no characterized proteins or genes are known.](http://</p>
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Enrichment cultures yielded bacterial isolates which could utilize *N*-methyltaurine as a sole source of carbon and energy or as a sole source of fixed nitrogen for growth. The data showed that the dissimilation of *N*-methyltaurine in *Alcaligenes faecalis* MT1 and in *Paracoccus versutus* N-MT proceeded via a cytochrome *c*-dependent oxidative removal of methylamine, to yield sulfoacetaldehyde, which was metabolized further.

METHODS

Materials. *N*-Methyltaurine (> 98 %) was purchased from Merck-Schuchardt. Other commercial chemicals (about 99 %) were from Fluka, Merck or Sigma-Aldrich. Solutions of taurine and *N*-methyltaurine were sterilized by autoclaving, which was not detrimental to these highly stable compounds. Fresh hypotaurine solutions were filter-sterilized through a 0.2 μ m filter (Schleicher & Schuell).

Organism, growth media, enrichment cultures with isolation of organisms, and preparation of cell-free extracts. *P. denitrificans* NKNIS (DSM 15418) and *P. pantotrophus* NKNCYSA (DSM 12449) were used as known sources of taurine dehydrogenase (Brüggemann *et al.*, 2004). All organisms were grown aerobically at 30 °C in batch culture in mineral-salts medium (Thurnheer *et al.*, 1986) with taurine, *N*-methyltaurine, acetate or methylamine

(10–20 mM), or with C₄–C₇ compounds (5 mM, see Results) as the sole added source of carbon and energy for growth. Negative controls without carbon source were also used. Nitrogen-limited batch cultures were grown aerobically at 30 °C in the salts medium modified by the omission of ammonium chloride and the addition of 20 mM acetate as carbon source; *N*-methyltaurine, taurine or ammonium ion (2 mM) served as sole added source of fixed nitrogen. Negative controls without a source of fixed nitrogen were also used. Precultures and cultures (3–5 ml) for the determination of the substrate range were grown in 30 ml screw-cap tubes in a roller. Growth experiments were done on the 100 ml scale in 1 litre Erlenmeyer flasks on a shaker: samples were taken at intervals to measure OD₅₈₀, to assay protein, and to determine the concentrations of substrate and products. Luria–Bertani (LB) agar plates (Sambrook *et al.*, 1989) were used during the enrichments of strains.

Cultures to enrich for organisms able to utilize *N*-methyltaurine as a sole source of carbon and energy contained 20 mM *N*-methyltaurine. They were inoculated with washed activated sludge from the aeration tank of the wastewater treatment plant in Konstanz, Germany, or with material from grassland soil or garden soils (from Switzerland and Germany) or littoral sediment from Lake Constance (Thurnheer *et al.*, 1986). Enrichment cultures with *N*-methyltaurine as the sole source of fixed nitrogen for growth, and the standard isolation procedure, were described elsewhere (Weinitschke *et al.*, 2005).

Cells were grown in 50 ml or 200 ml cultures in Erlenmeyer flasks, harvested, washed and ruptured by passage through a French pressure cell at 138 MPa; whole cells and debris were removed by centrifugation (10 min, 15 000 g, 4 °C) to yield crude extract (Denger *et al.*, 2004a). Membrane (particulate) and soluble fractions were obtained by ultracentrifugation (200 000 g, 30 min, 4 °C). The membrane fraction was resuspended in 50 mM phosphate buffer (pH 7.2) by the shear stress of being sucked into and expelled from a syringe.

Enzyme assays. *N*-Methyltaurine dehydrogenase was routinely assayed spectrophotometrically (600 nm) as the *N*-methyltaurine-dependent reduction of dichlorophenol indophenol [DCPIP; molar absorption coefficient 16 100 M⁻¹ cm⁻¹ (Jones, 1979)]. The reaction mixture contained (in a final volume of 1.0 ml): 50 µmol potassium phosphate buffer, pH 7.2, including 5 µmol MgCl₂, 25 µmol *N*-methyltaurine, 100 nmol DCPIP and 0.1–0.5 mg protein. The reaction was started by addition of *N*-methyltaurine, or of protein, and the response was linear ($R^2 > 0.99$) for at least 2 min. Enzyme activity in potassium phosphate buffer at pH 6.5, pH 7.0 or pH 7.6, or in Tris buffer at pH 9.0 (all 50 mM), was lower. The routine assay was occasionally augmented by following the formation of methylamine and sulfoacetaldehyde discontinuously. DCPIP could not be replaced with NAD⁺, but could be replaced with horse-heart cytochrome *c* (50 nmol); the wavelength used here was 555 nm and the molar absorption coefficient was 21 000 M⁻¹ cm⁻¹ (Ensley *et al.*, 1982) as described elsewhere (Brüggemann *et al.*, 2004). The assay for taurine dehydrogenase, analogous to that for *N*-methyltaurine dehydrogenase, was described previously (Brüggemann *et al.*, 2004). Sulfoacetaldehyde acetyltransferase (*Xsc*) was assayed by the colorimetric determination of acetyl phosphate or by the formation of sulfite (Ruff *et al.*, 2003). Phosphotransacetylase was assayed photometrically as the CoA-SH-dependent formation of acetyl-CoA (Bergmeyer *et al.*, 1983). Sulfite dehydrogenase was assayed spectrophotometrically with K₃Fe(CN)₆ or with cytochrome *c* as the electron acceptor (Reichenbecher *et al.*, 1999). Methylamine dehydrogenase was assayed as the methylamine-dependent reduction of K₃Fe(CN)₆ (Takagi *et al.*, 1999) based on the method of Eady & Large (1968). Enzyme activity is presented in the SI unit katal (kat, mol s⁻¹); thus 1 µmol min⁻¹ represents 16.7 nkat.

Analytical methods. Growth was followed as turbidity (OD₅₈₀) and quantified as protein in a Lowry-type reaction (Cook & Hütter,

1981). Reversed-phase chromatography was used to quantify *N*-methyltaurine, taurine, methylamine (Laue *et al.*, 1997) or free sulfoacetaldehyde (Cunningham *et al.*, 1998) after derivatization with 2,4-dinitrofluorobenzene or 2-(diphenylacetyl)indane-1,3-dione-1-hydrazone, respectively. 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). Formate was determined colorimetrically (Lang & Lang, 1972). The identification of the unknown peak as methylamine (as the dabsyl derivative) was confirmed by matrix-assisted, laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS): apparatus and methodology were as described elsewhere (Tholey *et al.*, 2002). Standard methods were used to establish the Gram reaction, and to assay catalase or cytochrome *c*-oxidase activity (Gerhardt *et al.*, 1994).

Molecular methods. Restriction enzymes, T4 DNA ligase and Taq DNA polymerase were from Fermentas and used as specified by the supplier. Oligonucleotides were synthesized by Hermann Gbr Synthetische Biomoleküle. In the oligonucleotide sequences Y = T + C, R = A + G, M = A + C, H = A + T + C, N = G + A + T + C and I = inosine, base-pairing with all four nucleotides. PCR products were purified with the E.Z.N.A. Cycle-pure kit (Peqlab Biotechnologie). Plasmid DNA was isolated using the E.Z.N.A. Plasmid purification kit I (Peqlab) if sequencing-grade DNA was required. Chromosomal DNA was isolated from bacteria as described by Desomer (1991) or by lysis at 95 °C according to Moore *et al.* (1996). Amplification of the 16S rRNA gene was done using the primers 16S-27F (5'-CAGAGT-TTGATCCTGGCTCAG-3') and 16S-1492R (5'-TACGGYTACCTGT-TACGACTT-3'); primers for sequencing were 16S-27F, 16S-1492R, 16S-533R (5'-TTACCGCGCTGCTGGCAC-3') and 16S-533F (5'-GTGCCAGCAGCCGCGGTAA-3') (Weisburg *et al.*, 1991).

PCR for *tauY* homologues was done with the degenerate PCR primers DegTauYF-Eco (5'-GCGAATTCGCICCIACITAYTG-3', *EcoRI* site underlined) and DegTauYR-Bam (5'-GTGGGGATCCTCNACCCA-ICCCCA-3', *BamHI* site underlined), while for *xsc* homologues the primers DegXscIIF (5'-GGAATTCGAYATHRAYTAYTTYC-3', *EcoRI* site underlined), DegXscI/IIIF (5'-GGAATTCGGNATNGAYTAYTG-GC-3', *EcoRI* site underlined) and DegXscR-Hin (5'-TTAAGCTTY-TCIGCICMCCA-3', *HindIII* site underlined) were used. Primers DegXscIIF and DegXscI/IIIF were used in a 1 : 2 mixture. The degree of degeneracy was kept to a minimum by the use of inosine residues. Amplification was done with an annealing temperature of 40 °C.

The following primers were used in *P. versutus* N-MT to sequence the *tauCXY-xsc-tauZ-pta* region: TauC-CYSA-1u, 5'-CCTTGGCCTATC-TGCCGCTG-3'; tauXF, 5'-ATCCACATATGACCCGCAACCTGA-3' (*NdeI* site underlined); tauXR, 5'-TGCGGATCCGCTCGTTTCTCGAT-3' (*BamHI* site underlined); tauYF, 5'-GGCCCCATATGCAGGCGAT-CGAGAA-3' (*NdeI* site underlined); tauYR, 5'-TCAGGATCCTGGC-GATGGCTCC-3' (*BamHI* site underlined); tauYcons1u, 5'-TAYGCG-CGACTACTGGRYCG-3'; tauYcons1l, 5'-CGCGGCATGATGTC-GTGGCTAC-3'; NMTtauYF, 5'-GGCGGCAACCGCGTGTATGAT-TC-3'; NMTxscR, 5'-GGATGCCGACCGTGACCTTCT-3'; NMTxscF, 5'-TCGAGGCCGGCGGAAATACC-3'; NMTtauZR, 5'-CATGGAT-GGTGCCGCCCAGAA-3'; tau-Z-f, 5'-GATTTCACCATGATCCC-TT-3'; and ptac-4l, 5'-ACCAGWCCGCARTCWGCRAAGA-3'. Primers were selected from a set designed to amplify the highly similar gene clusters from other *Paracoccus* spp. (Ruff *et al.*, 2003; Brüggemann *et al.*, 2004; T. H. M. Smits, unpublished).

PCR products were cloned into the vector pCR2.1 using the TA cloning kit (Invitrogen). Transformation of CaCl₂-competent *Escherichia coli* DH10B cells was done as described by Sambrook *et al.* (1989). Selection of transformants was done on LB agar containing 200 µg ampicillin ml⁻¹.

Sequencing was done using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Sequence runs were done at GATC Biotech. Sequences were analysed using the Lasergene package (DNASTar). Nucleotide and amino acid sequences were compared with the EMBL, Swissprot and GenBank databases using the BLAST server (Altschul *et al.*, 1997) at NCBI.

RESULTS

Enrichment cultures and isolates

Enrichment cultures, to test for the aerobic utilization of *N*-methyltaurine as a sole source of carbon and energy for growth of micro-organisms from activated sludge, grassland soil and garden soils, or from littoral sediment from Lake Constance, were successful. Subcultures grew overnight. The third subculture from the sludge inoculum was streaked on complex medium and found to be largely homogeneous; a representative colony was subcultured in selective liquid medium. Three cycles of plating on complex medium and growth in selective medium yielded strain MT1, which did not grow in the absence of *N*-methyltaurine and which showed similar growth in equimolar acetate-salts medium. Strain MT1 did not utilize *N*-methyltaurine as a sole source of nitrogen for growth, but could utilize taurine as a source of carbon or of nitrogen for growth.

Strain MT1 was a short, motile, Gram-negative rod ($0.5 \times 1-2 \mu\text{m}$), which was oxidase-positive and catalase-positive. The organism failed to grow with hypotaurine, isethionate, sulfoacetate, cysteate, glucose, fructose, galactose, adipate or salicylate, but did grow with taurine, acetate, benzoate, lactate or succinate. A 1390 bp fragment of the 16S rRNA gene was sequenced and found to share 98.7% identity of position with the 16S rRNA gene of the type strain of *Alcaligenes faecalis* (IAM12369^T) and 99.6% identity with *A. faecalis* WM2070 (Wilson & Metcalf, 2005). The physiological and nutritional properties of the organism (Holt *et al.*, 1994) confirmed this tentative identification, and the organism was deposited as a strain of *A. faecalis* with the German Culture Collection (DSMZ, Braunschweig, Germany) as DSM 16964.

Enrichment cultures to obtain aerobic organisms able to utilize *N*-methyltaurine as a sole source of fixed nitrogen for growth were also straightforward, and yielded strain N-MT. This organism could also dissimilate *N*-methyltaurine as a sole source of carbon for growth, and taurine served as a source of carbon, nitrogen or both.

Strain N-MT was a short, non-motile, Gram-negative rod ($0.7 \times 1-1.5 \mu\text{m}$), which was oxidase-positive and catalase-positive. The range of carbon substrates included formate, methanol, methylamine, glucose, maltose, malate, succinate, adipate and L-alanine, but not oxalate. A 1386 bp fragment of the 16S rRNA gene was found to share 99.8% identity of position with the type strain of *Paracoccus versutus* (ATCC 25364^T). The physiological (Holt *et al.*, 1994) and nutritional (Kelly *et al.*, 2000) properties of the organism

correspond to data on *P. versutus*, a species whose representatives were recognized mainly by 16S rRNA gene-sequence data (Rainey *et al.*, 1999).

Physiology of growth with *N*-methyltaurine

A. faecalis MT1 grew exponentially with *N*-methyltaurine as the sole source of carbon (not shown). The specific growth rate (μ) was 0.2 h^{-1} . Utilization of *N*-methyltaurine, determined by HPLC, was concomitant with growth (Fig. 2), and the molar growth yield (Y) was $9.6 \text{ g protein (mol } N\text{-methyltaurine)}^{-1}$. The release of a putative amine, detected as the dinitrophenyl derivative, was observed during growth (Fig. 2). The dinitrophenyl derivative of authentic methylamine co-eluted with the derivatized unknown. This tentative identification of the unknown as methylamine was confirmed by MALDI-TOF-MS, for which samples were derivatized by dabsylation. No derivative was obtained from freshly inoculated *N*-methyltaurine-salts medium, whereas outgrown medium contained a signal in the positive ion mode ($m/z = 319.3 = [M+1]^+$), which was identical to that of derivatized authentic methylamine ($M = 318$). The formation of methylamine was concomitant with growth (Fig. 2). The sulfonate moiety of the *N*-methyltaurine was recovered as sulfate, which was also released concomitant with growth (Fig. 2). Traces of sulfite were detected in the medium during growth (not shown). These data supported a degradative pathway involving (i) cleavage of methylamine from *N*-methyltaurine, with excretion of methylamine, and (ii) release of sulfite, which was largely oxidized to sulfate and excreted (Fig. 1).

P. versutus N-MT utilized 2 mM *N*-methyltaurine as a sole source of fixed nitrogen with transient excretion of up to 0.4 mM methylamine. The organism utilized methylamine quantitatively as a sole source of fixed nitrogen for growth: strain MT1 did not. No formate from methylamine was

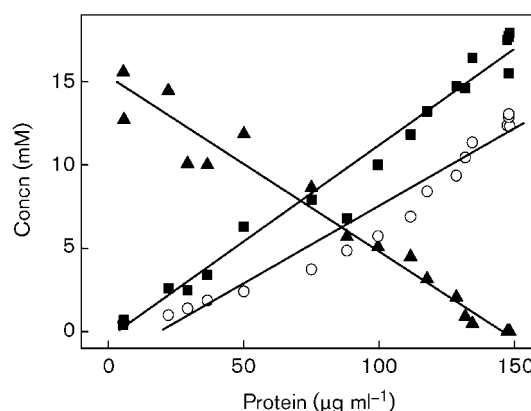


Fig. 2. Concentrations of substrate and products as a function of protein concentration during growth of *A. faecalis* MT1 in *N*-methyltaurine-salts medium. The data represent samples taken during 52 h of growth. ▲, *N*-Methyltaurine; ■, methylamine; ○, sulfate.

detected in the growth medium of strain N-MT. However, the organism utilized methylamine as a sole source of carbon and energy for growth, during which inducible methylamine dehydrogenase was synthesized (see below), so the methyl group of *N*-methyltaurine was presumably dissimilated via methylamine to cell material and CO₂ as elucidated for other *Paracoccus* spp. (Baker *et al.*, 1998). Strain N-MT excreted the sulfonate moiety of *N*-methyltaurine or taurine into the medium largely as sulfite, which was converted to sulfate, presumably chemically.

Known taurine degraders from our culture collection were tested for their ability to grow with *N*-methyltaurine. *Alcaligenes defragrans* NKNTAU, *P. denitrificans* NKNIS and *P. pantotrophus* NKNCYSA could utilize *N*-methyltaurine as a sole source of carbon and energy for growth. Both *Paracoccus* species could utilize *N*-methyltaurine as a sole source of nitrogen for growth and methylamine as a sole carbon source.

Enzyme reactions in the degradative pathway

The cleavage of methylamine from *N*-methyltaurine could theoretically be hydrolytic (see Introduction), but no substrate disappearance was detected in unamended cell extracts of *A. faecalis* MT1. A redox reaction analogous to taurine dehydrogenase (deaminating) (Brüggemann *et al.*, 2004) was tested. *N*-Methyltaurine dehydrogenase [0.4 mkat (kg protein)⁻¹] was detected photometrically with DCPIP as the electron acceptor. Methylamine and sulfoacetaldehyde (Fig. 1) were observed as products. No reaction was detected in extracts of acetate-grown cells (Table 1). *N*-Methyltaurine dehydrogenase could also be detected photometrically with cytochrome *c* as the electron acceptor, but at a lower specific activity [0.1 mkat (kg protein)⁻¹]. The preparation also contained taurine dehydrogenase activity (Table 1). *N*-Methyltaurine dehydrogenase was found in the membrane fraction, the location of the known taurine dehydrogenase (Brüggemann *et al.*, 2004).

Inducible taurine dehydrogenase activity, in the membrane fraction, was found in extracts of taurine-grown cells (Table 1) of *A. faecalis* MT1. These extracts also contained *N*-methyltaurine dehydrogenase activity.

Sulfoacetaldehyde acetyltransferase, which was not detected in extracts of acetate-grown cells of strain MT1, was detected at about 2.0 mkat (kg protein)⁻¹ in extracts of *N*-methyltaurine-grown cells (Table 1). The enzyme was found at a similar level [1.8 mkat (kg protein)⁻¹] in extracts of taurine-grown cells (Table 1). No phosphotransacetylase activity was detected.

No cytochrome *c*-coupled sulfite dehydrogenase was detected in extracts of *A. faecalis* MT1. However, a ferricyanide-coupled sulfite dehydrogenase was detected at significant levels in extracts of acetate-grown cells (Table 1), and eightfold higher levels [about 50 mkat (kg protein)⁻¹] were detected in extracts of *N*-methyltaurine- or taurine-grown cells.

Extracts of cells of *P. versutus* N-MT grown with *N*-methyltaurine as the sole source of fixed nitrogen contained *N*-methyltaurine dehydrogenase (and taurine dehydrogenase), sulfoacetaldehyde acetyltransferase and a ferricyanide-coupled sulfite dehydrogenase. The *N*-methyltaurine dehydrogenase was active with DCPIP. Extracts of the organism grown with acetate in the presence of excess ammonium ion did not contain these enzymes. No phosphotransacetylase activity was detected. Methylamine dehydrogenase could not be detected in *N*-methyltaurine-grown cells, but extracts of cells grown with methylamine as sole source of carbon and energy contained inducible methylamine dehydrogenase [13 mkat (kg protein)⁻¹].

Extracts of taurine-grown *P. denitrificans* NKNIS contained both taurine dehydrogenase activity [0.9 mkat (kg protein)⁻¹] and *N*-methyltaurine dehydrogenase activity [0.5 mkat (kg protein)⁻¹]. This observation led us to hypothesize that taurine dehydrogenase has a substrate range that includes *N*-methyltaurine. Extracts of taurine-grown and of *N*-methyltaurine-grown *P. pantotrophus* NKNCYSA similarly indicated the presence of inducible (*N*-methyl)taurine dehydrogenase; inducible Xsc was also observed.

Detection of genes involved in (*N*-methyl)taurine degradation

P. versutus N-MT is closely related phylogenetically to *P. pantotrophus* NKNCYSA, so primers previously designed for

Table 1. Specific activities of selected enzymes in extracts of cells of *A. faecalis* MT1 grown with acetate, taurine or *N*-methyltaurine as the sole source of carbon and energy

Carbon source	Specific activity [mkat (kg protein) ⁻¹]			
	<i>N</i> -Methyltaurine dehydrogenase	Taurine dehydrogenase	Sulfoacetaldehyde acetyltransferase	Sulfite dehydrogenase
Acetate	0	0	0	6 ± 0.02
Taurine	0.4 ± 0.02	0.4 ± 0.01	1.8 ± 0.02	45 ± 2
<i>N</i> -Methyltaurine	0.4 ± 0.004	0.4 ± 0.01	2.0 ± 0.17	53 ± 3

the latter strain and other *Paracoccus* spp. were used to amplify and sequence the *tauCXY-xsc-tauZ-pta* gene cluster. Two contiguous sequences were obtained (*tauCXY-xsc* and *tauZ-pta*), with a 70 bp portion of the intergenic region between *xsc* and *tauZ* missing. Sequencing this region in other organisms was repeatedly difficult (J. Ruff & A. M. Cook, unpublished), probably due to the strong inverted repeats seen in *P. pantotrophus* strains NKNCYSA and DSM65 and *P. denitrificans* strains NKNIS and PD1222, so no further effort was made to sequence the amplicon representing the *xsc-tauZ* genes.

Sequence identities between the *P. versutus* N-MT and *P. pantotrophus* NKNCYSA regions were high. Overall, the *P. versutus* N-MT *tauCXY-xsc* region had 99.1% and 93.3% sequence identity with the corresponding regions in *P. pantotrophus* strains NKNCYSA and DSM65, respectively; the *tauZ-pta* region had 99.4% and 93.3% sequence identity with the corresponding regions in *P. pantotrophus* strains NKNCYSA (where available) and DSM65 (full-length), respectively. The proteins are also highly conserved between the two *Paracoccus* species (strains N-MT and NKNCYSA): TauC (C-terminus in N-MT) 100%; TauX 98.6%; TauY 98.7%; Xsc 99.8%; TauZ (N-terminus in NKNCYSA) 98.5%. The *pta* gene of NKNCYSA was not sequenced, although based on PCR fragments, it is also located immediately downstream of *tauZ* (J. Ruff & T. H. M. Smits, unpublished). The partial amino acid sequence of Pta from strain N-MT shares 92.8% identity with the corresponding protein in *P. pantotrophus* DSM65.

A different approach had to be taken to sequence a portion of the *tauXY* genes presumed to encode the (*N*-methyl)taurine dehydrogenase from *A. faecalis* MT1 (see Introduction and above). We developed, from the available sequences, degenerate PCR primers which would allow the amplification of a large portion of the *tauY* gene. An amplicon of the correct size (1050 bp) was obtained from *A. faecalis* MT1 and sequenced. The derived amino acid sequence shared 78% identity of position with the corresponding fragment of the derived TauY protein from *Cupriavidus necator* JMP134 (see Brüggemann *et al.*, 2004). Similarly, PCR was done with degenerate *xsc*-gene primers which target a 644 bp internal fragment of the gene. The primers were designed to complement sequence motifs in all three subgroups of Xsc sequences (cf. Ruff *et al.*, 2003), and therefore were based on partially different sequences, but all at the same position. An amplicon of about 650 bp was obtained and sequenced. The derived peptide sequence shared more than 75% identity of position with the corresponding fragments of Xsc homologues in subgroup 1, which contains other β -Proteobacteria and high-GC Gram-positive bacteria (Ruff *et al.*, 2003; Brüggemann *et al.*, 2004).

DISCUSSION

The widespread and diverse metabolism of the osmolyte taurine (Cook & Denger, 2006) led us to expect that the

chemically similar osmolyte *N*-methyltaurine would also be biodegradable. The hypothesis was confirmed. All tested inocula gave positive enrichments (see above) while the known taurine-degraders *A. defragrans* NKNTAU, *P. pantotrophus* NKNCYSA, *P. denitrificans* NKNIS (see above) and *S. pomeroyi* DSS-3 (K. Denger, unpublished) could also utilize *N*-methyltaurine as a sole source of carbon, which suggested that degradation is widespread. We wonder whether *N*-methyltaurine is more widespread than indicated by its presence in many marine animals (Allen & Garrett, 1971) and its known role as an osmolyte in deep-sea worms (Yancey *et al.*, 2002).

The growth experiment (Fig. 2) showed that *A. faecalis* MT1 utilizes *N*-methyltaurine quantitatively with the release of 1.1 mol methylamine (mol *N*-methyltaurine)⁻¹ and 0.9 mol sulfate (mol *N*-methyltaurine)⁻¹. We interpret this to mean unit stoichiometry: this led to the hypothesis in Fig. 1. The hypothesized enzymes are expressed inducibly (Table 1). The data show that the pathway in *P. versutus* N-MT is similar to that in Fig. 1, including transient excretion of methylamine; but *P. versutus* N-MT contains, in addition, methylamine dehydrogenase, which allows it to release the amine as the ammonium ion and thus assimilate the nitrogen from *N*-methyltaurine.

The first metabolic enzyme in the pathway (Fig. 1) catalyses a novel reaction, *N*-methyltaurine dehydrogenation, which is presumed to be catalysed by taurine dehydrogenase. The (*N*-methyl)taurine dehydrogenase from *A. faecalis* MT1 showed a much higher activity with the artificial electron acceptor, DCPIP, than with equine cytochrome *c*, as observed with some other taurine dehydrogenases (Brüggemann *et al.*, 2004). One taurine dehydrogenase, postulated from the presence of the *tauXY* genes in *Rhodospseudomonas palustris* CGA009, is inactive with both DCPIP and equine cytochrome *c* (Denger *et al.*, 2004b), which is itself an artificial substrate in bacterial extracts. However, S. Weinitschke (unpublished data) has now observed that the addition of separated cytochrome *c* (from extracts of taurine-grown *R. palustris*) to the apparently inactive crude extract from *R. palustris* allows oxidation of taurine to sulfoacetaldehyde. This tends to confirm the hypothesis of Denger *et al.* (2004b) that a native cytochrome *c* is sometimes essential for activity of taurine dehydrogenase. Correspondingly, characterization of taurine dehydrogenase will require not only pure protein (not attained elsewhere; Brüggemann *et al.*, 2004) but also its native cytochrome *c*.

The hypothesis that TauXY catalyses the (*N*-methyl)taurine dehydrogenase reaction led us to discover *tauY*-like genes in *A. faecalis* MT1 and *P. versutus* N-MT. This emphasizes the importance of the use of degenerate PCR primers to locate genes with high sequence similarity and, presumably, iso-functional gene products (e.g. Smits *et al.*, 1999; van Beilen *et al.*, 2003; Regeard *et al.*, 2004). The presence of *tauXY* genes predicts the presence of taurine dehydrogenase in all cultures available to us, and the presence of taurine dehydrogenase predicts the presence of *tauXY* genes, but there is

still no direct proof that TauXY is taurine dehydrogenase. We have been unable to express the *tauXY* genes from *P. versutus* N-MT in *E. coli* (T. H. M. Smits, unpublished).

The degradative pathway for *N*-methyltaurine in *A. faecalis* MT1 proceeds via sulfoacetaldehyde acetyltransferase (Xsc) (Table 1), which releases sulfite (Fig. 1). This compound is oxidized to sulfate by the undefined sulfite dehydrogenase which is assayed with ferricyanide as the electron acceptor (Table 1), and the sulfate is excreted. The mechanism of excretion is unknown, but candidate gene products (TauZ in α -Proteobacteria and OrfX in β -Proteobacteria) are known in other systems (Rein *et al.*, 2005). The mechanism of excretion of methylamine (Figs 1 and 2) is also unknown, but might be performed by the ammonium-methylamine transporter AmtB (Khademi *et al.*, 2004; Booth *et al.*, 2005).

The degradative pathway for *N*-methyltaurine in *P. versutus* N-MT shares many facets with the pathway in Fig. 1. The facts that the taurine gene cluster from *P. pantotrophus* NKNCYSA is effectively identical to the cluster in strain N-MT, and that strain NKNCYSA shares the ability to dissimilate *N*-methyltaurine via (*N*-methyl)taurine dehydrogenase, allow us to use sequence data from both organisms to confirm and expand on the enzymic data, and to predict some membrane functions in Fig. 1. An ABC transporter (TauABC) pumps (*N*-methyl)taurine into the cell. TauZ functions as a sulfite/sulfate exporter. The genes (*tauXY*) putatively encoding taurine dehydrogenase and a candidate *xsc* gene are present. A *pta* gene is available (to yield acetyl-CoA) but we could not detect Pta activity, so this gene product is presumably unstable in cell extracts. The Krebs cycle can be presumed in the organisms, although different anaplerotic pathways may be anticipated in the α - and β -Proteobacteria (e.g. Novak *et al.*, 2004). A methylamine exporter seems to be present in strain N-MT, as methylamine appears transiently in the growth medium, but the significant metabolic difference between the organisms is the ability of *P. versutus* N-MT to cleave the C–N bond of methylamine, to make the nitrogen available for growth.

A more subtle difference between strains MT1 (Fig. 1) and N-MT is the rare, extensive excretion of sulfite by strain N-MT. This phenomenon, which is more extreme in *S. pomeroyi* DSS-3, is discussed elsewhere (Denger *et al.*, 2006).

ACKNOWLEDGEMENTS

We are grateful to Anja Dahler, Matthias Erhardt, Michael Fehlings, Christoph Goletz, Christoph Rockel and Anke Schwarzenberger for data generated in a practical class for advanced undergraduates. The research was funded by the University of Konstanz and the LBS-Stiftung 'Umwelt und Wohnen'.

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