

Dissimilation of cysteate via 3-sulfolactate sulfo-lyase and a sulfate exporter in *Paracoccus pantotrophus* NKNCYSA

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Paracoccus pantotrophus NKNCYSA utilizes (*R*)-cysteate (2-amino-3-sulfo-propionate) as a sole source of carbon and energy for growth, with either nitrate or molecular oxygen as terminal electron acceptor, and the specific utilization rate of cysteate is about 2 mkat (kg protein)⁻¹. The initial degradative reaction is catalysed by an (*R*)-cysteate : 2-oxoglutarate aminotransferase, which yields 3-sulfo-pyruvate. The latter was reduced to 3-sulfolactate by an NAD-linked sulfolactate dehydrogenase [3.3 mkat (kg protein)⁻¹]. The inducible desulfonation reaction was not detected initially in cell extracts. However, a strongly induced protein with subunits of 8 kDa (α) and 42 kDa (β) was found and purified. The corresponding genes had similarities to those encoding altronate dehydratases, which often require iron for activity. The purified enzyme could then be shown to convert 3-sulfolactate to sulfite and pyruvate and it was termed sulfolactate sulfo-lyase (Suy). A high level of sulfite dehydrogenase was also induced during growth with cysteate, and the organism excreted sulfate. A putative regulator, OrfR, was encoded upstream of *suyAB* on the reverse strand. Downstream of *suyAB* was *suyZ*, which was cotranscribed with *suyB*. The gene, an allele of *tauZ*, encoded a putative membrane protein with transmembrane helices (COG2855), and is a candidate to encode the sulfate exporter needed to maintain homeostasis during desulfonation. *suyAB*-like genes are widespread in sequenced genomes and environmental samples where, in contrast to the current annotation, several presumably encode the desulfonation of 3-sulfolactate, a component of bacterial spores.

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INTRODUCTION

(*R*)-Cysteate (L-cysteate; 2-amino-3-sulfo-propionate, Fig. 1) is a natural product first found in wool, and it is now known as an intermediate in cysteine metabolism involved in, for example, the synthesis of taurine (2-aminoethanesulfonate) in mammals (Consden *et al.*, 1946; Weinstein & Griffith, 1988). The compound also serves as a precursor of the cytophagal sulfolipid capnine (White, 1984) and is found extracellularly in spiders' webs (Vollrath *et al.*, 1990). Cysteate serves as a sole source of carbon and energy for the aerobic growth of bacteria (Stapley & Starkey, 1970), as an electron acceptor for several sulfate-reducing bacteria (Lie *et al.*, 1996, 1998, 1999; Laue *et al.*, 1997a), as an electron donor for some nitrate-reducing bacteria (Denger *et al.*, 1997; Mikosch *et al.*, 1999), and as the substrate for a

fermentation in a sulfate-reducing bacterium (Laue *et al.*, 1997b). None of those papers reports a degradative pathway, but Mikosch *et al.* (1999) did observe 2-oxoglutarate-dependent transamination of cysteate to 3-sulfo-pyruvate (EC 2.6.1.–) in *Paracoccus pantotrophus* NKNCYSA and regeneration of the 2-oxoglutarate (EC 1.4.1.4) (Fig. 1). Neither taurine dehydrogenase (presumably EC 1.4.2.–) nor sulfoacetaldehyde acetyltransferase (EC 2.3.3.15), which are induced in *P. pantotrophus* NKNCYSA during dissimilation of taurine (Ruff *et al.*, 2003; Brüggemann *et al.*, 2004), is observed during growth with cysteate (K. Denger, unpublished), so the major cysteate transformation known in mammalian tissue, decarboxylation to taurine (Weinstein & Griffith, 1988), is not used in *P. pantotrophus*.

3-Sulfo-pyruvate and 3-sulfolactate are established natural products (e.g. Bonsen *et al.*, 1969; Graupner *et al.*, 2000a) whose degradative pathway in prokaryotes is unknown (e.g. Roy *et al.*, 2003). Sulfo-pyruvate is subject to reduction to (*S*)-sulfolactate by 3-sulfolactate dehydrogenase (EC 1.1.1.272) in, for example, the biosynthesis of coenzyme

The GenBank/EMBL/DDBJ accession numbers for the sequences in *P. pantotrophus* NKNCYSA reported in this paper are: *orfR* and *suyABZ*, AY704413; extension of the gene sequence in the *tau* region to include most of *tauZ*, AY498615.

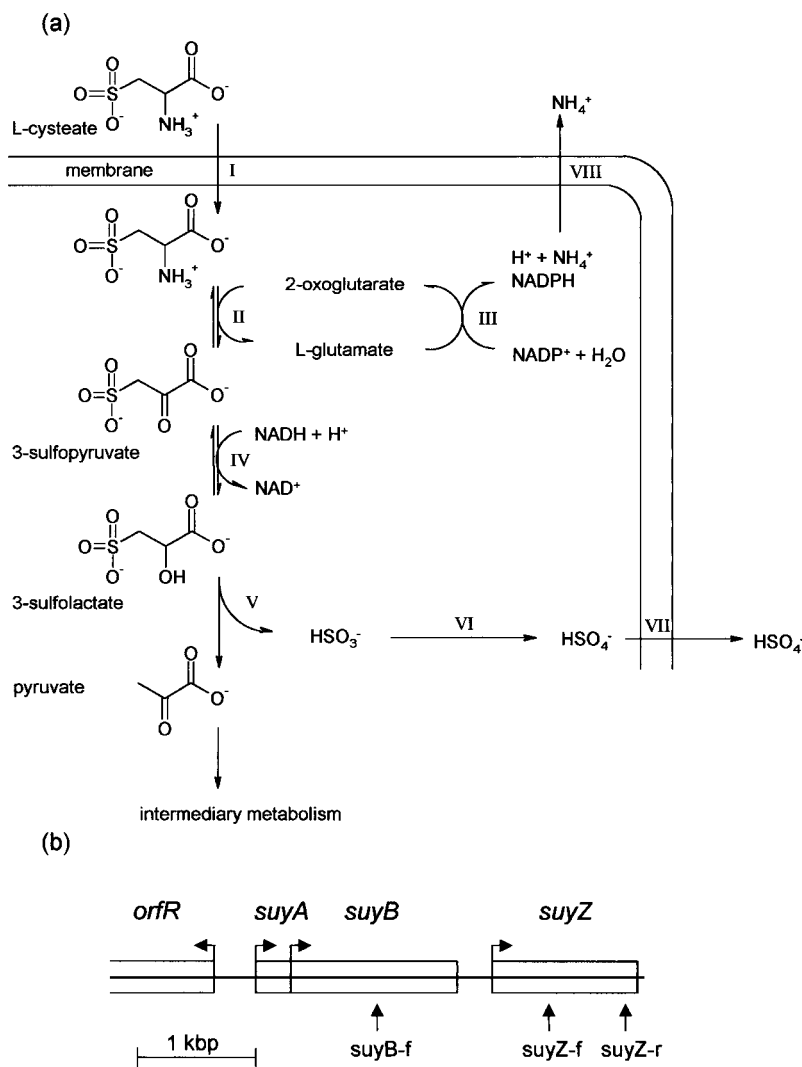


Fig. 1. Transport and biochemical reactions involving sulfonated compounds in the degradative pathway of cysteate (a) and the organization of the cluster of *suy* genes (b) in *P. pantotrophus* NKNCYSA. This pathway, in which initially only reactions II and III were established (Mikosch *et al.*, 1999), was developed as a working hypothesis when a partial sequence of *suyB* became available (see text); the components of ionic homeostasis were developed from earlier work (Brüggemann *et al.*, 2004). Reaction I is an as yet undefined transport system. Reaction II is L-cysteate:2-oxoglutarate aminotransferase. Reaction III is L-glutamate dehydrogenase. Reaction IV is sulfolactate dehydrogenase. Reaction V is sulfolactate sulfo-lyase (SuyAB). Reaction VI is sulfite dehydrogenase (EC 1.8.2.1). Reaction VII is sulfate export, and reaction VIII represents export of the ammonium ion. The gene cluster (b), which was discovered and sequenced in this work, involves at least four open reading frames, encoding a putative transcriptional regulator (OrfR), the sulfolactate sulfo-lyase (SuyAB) and the presumed sulfate exporter (SuyZ). The binding sites of the primers used for RT-PCR are shown with arrows.

M (Graupner *et al.*, 2000a). The reaction can also be catalysed by mammalian malate dehydrogenase (EC 1.1.1.37), where the resulting sulfolactate is excreted (Weinstein & Griffith, 1986, 1988), but 3-sulfolactate dehydrogenase represents a specific lineage within the family of malate dehydrogenases (Graupner *et al.*, 2000b). Sulfolactate is widespread as a component of bacterial endospores (Bonsen *et al.*, 1969; Wood, 1971; see also Graham *et al.*, 2002) and is now known to be an intermediate in one pathway of degradation of sulfoquinovose (Roy *et al.*, 2003).

Our initial hypothesis for the desulfonation reaction in cysteate degradation in the nitrate-reducing strain NKNCYSA was based on the only characterized, non-oxygenolytic mechanism known, catalysed by sulfoacetaldehyde acetyltransferase (EC 2.3.3.15). This hypothetical, thiamin-diphosphate-dependent reaction would involve sulfolactate, and would yield acetyl phosphate. There was, however, an inducible protein of unknown function in extracts of cysteate-grown cells. This protein was purified, and the corresponding gene was sequenced. Sequence similarity to the enolase superfamily allowed the reaction

mechanism of sulfolactate sulfo-lyase (Suy) and the overall pathway to be hypothesized (Fig. 1). The hypothesis was tested in this paper.

The degradative pathways of cysteate and taurine are independent of one another, but they share poorly understood characteristics. Growth with each compound as a carbon source is accompanied by excretion of both ammonium and sulfate ions (Mikosch *et al.*, 1999; Brüggemann *et al.*, 2004) (Fig. 1). These are presumably homeostatic mechanisms to maintain constant ionic conditions within the cell, and neither process is understood (Brüggemann *et al.*, 2004; see also Denger *et al.*, 2004).

Evidence is now presented that the desulfonative pathway of the C₃-sulfonates cysteate, sulfolactate and sulfolactate in *P. pantotrophus* NKNCYSA involves inducible sulfolactate sulfo-lyase (SuyAB), a novel member of the altronate dehydratases, orthologues of which seem to be encoded on many genomes. It is further proposed that the gene (*suyZ*) downstream of *suyAB* encodes a sulfate exporter, which is also widespread.

METHODS

Materials. (*R*)-Cysteic acid monohydrate (>99%) was from Fluka. Authentic 3-sulfolactate was kindly supplied by R. H. White (cf. White, 1986). 3-Sulfolactate (Denger *et al.*, 2001) and 3-sulfolactate (Roy *et al.*, 2003) were synthesized and identified as described elsewhere. The sulfolactate had a specific optical rotation of -0.7° , whereas the literature values for D- and L-sulfolactate are $+3.5^\circ$ and -3.1° , respectively (Graham & White, 2002); our synthetic material was effectively racemic. Commercial chemicals were of high purity (about 99%), from Sigma-Aldrich, Fluka or Merck and they were used as supplied by the manufacturer. Sulfoacetate was from Fisher Scientific. Gases (N_2 , CO_2) were obtained from Messer Griesheim.

Growth media and organisms used. Most experiments were done with bacteria grown in basal salts medium representing fresh water, buffered with 50 mM $NaHCO_3$, and prepared anoxically as described by Widdel & Pfennig (1981). The supplements and alterations used to provide conditions for nitrate reduction, sulfonate reduction or fermentation were described elsewhere (Laue *et al.*, 1997b; Mikosch *et al.*, 1999). *P. pantotrophus* NKNCYSA (DSM 12449) (Mikosch *et al.*, 1999), *Desulfovibrio* sp. strain RZACYSA, *Bilophila wadsworthia* RZATAU (DSM 11045) (Laue *et al.*, 1997a) and *Desulfovibrio* sp. strain GRZCYSA (DSM 11493) (Laue *et al.*, 1997b) were used. *Desulfobacterium hafniense* DCB-2 was kindly made available by J. Tiedje, East Lansing, MI, USA. Strain DCB-2 grew with 3-sulfolactate as the electron acceptor and lactate as the electron donor when the medium was supplemented with 0.1% yeast extract. The growth medium for cells grown under oxic conditions is indicated in Brüggemann *et al.* (2004). *Cupriavidus necator* (*Ralstonia eutropha*) JMP134 (DSM 4058) (Vandamme & Coenye, 2004) and *Novosphingobium aromaticivorans* (DSM 12444^T) were obtained from the German Culture Collection (DSMZ, Braunschweig, Germany).

Permeabilized cells. Cell suspensions ($0.4 \text{ mg protein ml}^{-1}$, 1 ml) were incubated for 10 min at 37°C in 0.1 M Tris/HCl buffer, pH 7.5, which contained 5 mM $MgCl_2$ and cetyltrimethylammonium bromide [$0.25 \text{ mg (mg protein)}^{-1}$] before the reaction was started with the addition of 1–2 mM substrate. Thereafter, samples were taken at intervals to determine concentrations of substrate and of possible products.

Preparation of cell-free extracts and nucleic acids. Cells for the preparation of cell-free extract were harvested (13 000 g, 20 min, 4°C) at the end of the exponential growth phase and disrupted by three passages through a chilled French pressure cell (140 MPa). Whole cells and debris were removed by centrifugation (5000 g, 20 min, 4°C) to give the crude cell extract. Soluble and membrane fractions of the crude extract were obtained by ultracentrifugation (150 000 g, 2 h, 4°C). Nucleic acids were precipitated as described elsewhere (Ruff *et al.*, 2003).

Enzyme measurements. (*R*)-Cysteate:2-oxoglutarate aminotransferase was assayed discontinuously as disappearance of cysteate (Mikosch *et al.*, 1999). Glutamate dehydrogenase (EC 1.4.1.4) was assayed photometrically (Schmidt, 1974). 3-Sulfolactate dehydrogenase was routinely assayed photometrically (340 nm) as sulfolactate-dependent oxidation of NADH: the reaction mixture (1 ml final volume) contained 50 $\mu\text{mol Tris/HCl}$ buffer, pH 7.5, 0.25 $\mu\text{mol NADH}$, 2 $\mu\text{mol sulfolactate}$ and 15–30 $\mu\text{g protein}$, with which the reaction was started. Occasionally, the reaction mixture was used to follow the conversion of sulfolactate to sulfolactate by ion chromatography. 3-Sulfolactate sulfo-lyase was assayed discontinuously at 30°C as disappearance of racemic 3-sulfolactate and formation of sulfite and pyruvate. The reaction mixture (2 ml) contained 200 $\mu\text{mol MOPS/NaOH}$ buffer, pH 6.5, 2 $\mu\text{mol FeCl}_2$, 5 μmol

sulfolactate and 2–4 mg protein, with which the reaction was started. Samples were taken at intervals: (a) 200 μl to acetonitrile (100 μl) for the determination of sulfolactate by ion chromatography; (b) 50 μl to solution A (950 μl) (Denger *et al.*, 2001) for the determination of sulfite as the fuchsin adduct; (c) 50 μl to 2 M HCl (10 μl) for the enzymic quantification of pyruvate after removal of protein by centrifugation (2 min, 20 000 g, room temperature). Sulfite dehydrogenase (EC 1.8.2.1) was assayed photometrically with cytochrome *c* as electron acceptor (Reichenbecher *et al.*, 1999). Pyruvate disappearance was assayed discontinuously at 37°C : the reaction mixture (1 ml final volume) contained 100 $\mu\text{mol potassium phosphate buffer}$, pH 7.5, 2–4 mg protein and 2 $\mu\text{mol pyruvate}$, with which the reaction was started. Samples (100 μl) were taken at intervals into 20 μl 2 M HCl and the concentration of pyruvate was determined after derivatization.

Protein purification. Anion-exchange chromatography was done as described previously (Denger *et al.*, 2001). The buffer was modified to include 10 $\mu\text{M pyridoxal 5'-phosphate}$. Each fraction was assayed for (*R*)-cysteate:2-oxoglutarate aminotransferase, sulfite dehydrogenase and glutamate dehydrogenase; the presence of the 42 kDa protein was detected by SDS-PAGE.

The fraction which contained the 42 kDa protein was concentrated, rebuffed and subjected to hydrophobic interaction chromatography on Phenyl Superose HR (5/5 column; Pharmacia). A linear decreasing gradient from 1.7 M ammonium sulfate in 20 mM Tris/sulfate, pH 7.5, and containing 10 $\mu\text{M pyridoxal 5'-phosphate}$, was applied. The 42 kDa protein eluted at 0 M ammonium sulfate.

Analytical methods. The HPLC system was equipped with a diode array detector, and used for reverse-phase chromatography (Laue *et al.*, 1996). Cysteate was quantified by HPLC after derivatization with 2,4-dinitrofluorobenzene (DNFB) (Sanger, 1945) as described elsewhere (Denger *et al.*, 1997). Pyruvate was routinely derivatised with 2,4-dinitrophenylhydrazine, and measured photometrically at 450 nm; pyruvate was also determined enzymically with lactate dehydrogenase (Lamprecht & Heinz, 1984). Pyruvate was identified by MALDI-TOF MS after derivatization (Tholey *et al.*, 2002). 3-Sulfolactate was determined as the azine formed by reaction with 2-(diphenylacetyl)indane-1,3-dione-1-hydrazone (Cunningham *et al.*, 1998) after separation by HPLC with UV detection (400 nm). A perchlorate mobile phase with a gradient of acetonitrile (30–80%) was used (cf. Schleich *et al.*, 2000). Nitrate, nitrite and sulfoacetate, and occasionally sulfolactate, sulfolactate, 2-oxoglutarate, glutamate, sulfate and sulfite, were separated on an LCA 3AS or A03 column by ion chromatography with suppression and quantified in a conductivity detector (Laue *et al.*, 1996). Sulfate was measured as the attenuation of a suspension of insoluble $BaSO_4$ (Sörbo, 1987). Sulfite was quantified photometrically as the fuchsin adduct (Denger *et al.*, 2001).

Protein in whole cells was assayed by a Lowry-type reaction (Kennedy & Fewson, 1968). 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 masses of bands were determined by comparison with standard proteins (Low Range Marker Proteins, Bio-Rad). The N-terminal amino acid sequence of a blotted protein was determined after Edman degradation, as indicated previously (Schläfli *et al.*, 1994). The sequences of internal peptides were obtained by Edman degradation after proteolysis (Lys C) of the separated protein and separation of the peptides by HPLC (Top-Lab Service Facility, München, Germany). Total bacterial DNA was prepared by phenol/chloroform extraction (Ausubel *et al.*, 1987). The DNA sequence of the putative 3-sulfolactate sulfo-lyase genes, with those of the upstream and downstream genes (Fig. 1b), was generated by PCR, cycle sequencing (BigDye ABI-technology at GATC, Konstanz), primer

walking and the GenomeWalker approach (BD Biosciences, Clontech) (Denger *et al.*, 2001; Tralau *et al.*, 2003b). The initial PCR-primers were suy1-f, TTYWSIAAYGCIACIGTIARGC; suy2-f, ACGCAGG-TGATCGTCGACG; suy3-r, CKDATIGTYTCRAARTCICC; suy4-r, ACITTYTCYTCYTCDATNGTNGTIARICCI. Sequence analysis was done using the DNASTAR Lasergene program package. The NCBI BLAST programs (<http://www.ncbi.nlm.nih.gov/blast/>), or analogues in the TIGR website (<http://www.tigr.org/tdb/mdb/mdbinprogress.html>) and the Transport Classification Database (TCDB) (<http://tcdb.ucsd.edu/tcdb/>), were used to search for similarities amongst the new and established sequences (Altschul *et al.*, 1997). The PROSITE tools of the Swiss Institute of Bioinformatics were used to search for motifs in the SWISS-PROT database on the ExPASy server (<http://www.expasy.org/>). The predictions of transmembrane helices were generated by the TMHMM server (<http://www.cbs.dtu.dk/services/>). Reverse-transcription PCR (RT-PCR) was done as described previously (Tralau *et al.*, 2003a), with 0.4 µg total RNA from cultures in the mid-exponential phase of growth; the Taq polymerase was from Genaxxon. The following primers were used: suyZ-f, GATCAT-CAGGACGCGCGGGA; suyZ-r, ACCAGGACGACAGCGCGGGA; tauZ-f: TCCACCATGATCCCTTCGC; tauZ-r: AAAGCCCGGCCA-GCATCGGG; suyB-f; ACCAAGGGCAACATTCTTGG. The PCR programme consisted of 10 cycles with an initial annealing temperature of 60 °C and a decrement of 0.5 °C (touch-down) followed by 25 cycles with an annealing temperature of 57 °C; the extension time was 1 min at 72 °C.

The sequence data on *Burkholderia xenovorans* LB400, *Burkholderia* sp. R18194 (*cepacia*-like), *Cupriavidus necator* JMP134, *Desulfotobacterium hafniense* DCB-2, *Novosphingobium aromaticivorans* SMCC F199, *Rhodobacter sphaeroides* 2.4.1, *Rubrobacter xylanophilus* DSM 9941 and *Trichodesmium erythraeum* IMS101 were generated by the US Department of Energy's Joint Genome Institute and are publicly available at <http://www.jgi.doe.gov>. Sequence data on *Silicibacter pomeroyi* DSS-3^T, and information on funding the project, were obtained from The Institute for Genomic Research through the website at <http://www.tigr.org>. The accession number for clone P1023 in the *Leishmania major* genome project is AC091510. The other genomic sequences were from published data.

RESULTS

Chemically defined reactions from cysteate to pyruvate and sulfate

The presence of cysteate:2-oxoglutarate aminotransferase and of NADP-coupled glutamate dehydrogenase in extracts of cells of *P. pantotrophus* NKNCYSA grown with cysteate under anoxic conditions was confirmed (Mikosch *et al.*, 1999) and found in addition in cells grown under oxic conditions (not shown). These extracts also caused disappearance of 3-sulfo-pyruvate, in the presence of NADH, and the appearance of a compound which co-chromatographed with authentic 3-sulfolactate in an ion chromatograph. Boiled extract caused no reaction. The specific activity of the sulfolactate dehydrogenase was 42 mkat (kg protein)⁻¹. Each of these three enzymes was present at high levels under all conditions measured (e.g. acetate-grown and cysteate-grown cells) (not shown).

Whole cells of cysteate-grown *P. pantotrophus* did not degrade 3-sulfo-pyruvate, whereas permeabilized cells did so. We presume that the organism has no transport system

for 3-sulfo-pyruvate, and that the cell membrane is impermeable to organosulfonates (see Graham *et al.*, 2002). The organism obviously has a transport system for cysteate, because it utilizes cysteate, and a transport system for sulfolactate, which it also utilizes.

Whole cells or permeabilized cells of *P. pantotrophus* released equimolar sulfate from the portion of sulfolactate that disappeared (not shown). No sulfite was detected. Initially, no disappearance of the substrate was detected in crude extracts, but we developed the hypothesis that an enolase-like reaction was catalysed (sulfolactate sulfo-lyase: reaction V in Fig. 1; see also Discussion) and altered the reaction mixture accordingly. Crude extract then converted sulfolactate to compounds which were tentatively identified as sulfite and pyruvate (Fig. 2). The stoichiometry was essentially 1:1:1 (Fig. 2): in longer experiments (not shown), the formation of sulfate was detected. The identity of the sulfite, routinely assayed as an adduct of fuchsin, was confirmed by co-chromatography with authentic material in an ion chromatograph. The tentative identification of the pyruvate, initially determined in the specific reaction catalysed by lactate dehydrogenase, was supported by the colour reaction with 2,4-dinitrophenylhydrazine. The identification of pyruvate was confirmed by MALDI-TOF MS after reaction with 1,2-phenylenediamine as the quinoxalinol derivative in the positive-ion mode ($m/z = 161 [M + H]^+$).

Sulfolactate sulfo-lyase was found in cysteate- and sulfolactate-grown cells only. None was found in extracts of acetate-, taurine-, isethionate- or sulfoacetate-grown cells. Sulfolactate sulfo-lyase was considered to be inducible.

The reaction in Fig. 2 had largely ceased within 30 min, with about 50% of the substrate remaining. When more substrate was added, a rapid reaction was observed which largely ceased when 50% of the added material remained. Thereafter, there was a slow disappearance of substrate.

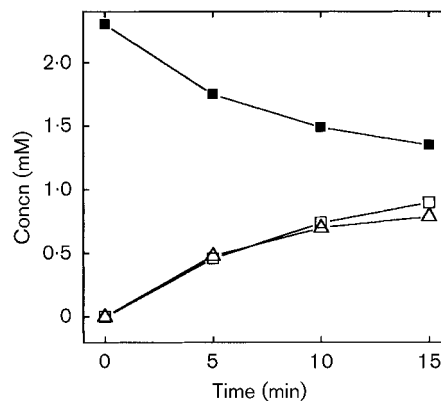


Fig. 2. Transformation of 3-sulfolactate in crude extract of *P. pantotrophus* NKNCYSA. ■, 3-sulfolactate; △, sulfite; □, pyruvate.

Racemic sulfolactate was synthesized chemically, and it was inferred that (i) sulfolactate sulfo-lyase is enantiomer-specific, and (ii) a sulfolactate racemase is present at low activity.

The requirement of sulfolactate sulfo-lyase for Fe^{2+} was examined. No requirement for supplementary iron was detected in crude extract or in desalted crude extract. However, when desalted crude extract was preincubated (10 min) with EDTA (1 mM final concentration), >70% of the enzyme activity was lost. Readdition of Fe^{2+} (or Mn^{2+} or Co^{2+} to 2 mM final concentration) led to a full recovery of activity.

The inorganic product released from sulfolactate sulfo-lyase was sulfite. Whole cells, however, released only sulfate. Further, cell extracts containing sulfolactate sulfo-lyase soon contained sulfate after addition of sulfolactate, so we tested for a sulfite dehydrogenase in crude extract, and found the enzyme in sufficient activity [$3.3 \text{ mkat} (\text{kg protein})^{-1}$] to explain the absence of sulfite in the growth medium. The sulfite dehydrogenase was present in cells expected to release sulfite (cysteate and sulfoacetate) and absent in acetate-grown cells. The enzyme was presumed to be inducible.

Pyruvate was dissimilated by extracts of cells of *P. pantotrophus* grown with cysteate or acetate. The metabolism of pyruvate is presumed to be constitutive, which would allow any pyruvate from C_3 -sulfonates to be dissimilated.

Purification and identification of sulfolactate sulfo-lyase in *P. pantotrophus*

When proteins in extracts of individual species of bacteria grown with different organosulfonates are separated on SDS-PAGE gels, patterns of proteins specific for each substrate can be observed (Lie *et al.*, 1998; Denger & Cook, 2001). Extracts of *P. pantotrophus* grown with acetate, cysteate, sulfolactate (Fig. 3, lanes 1–3), sulfoacetate or taurine (not shown) were compared. One major difference was immediately obvious. Only the extracts from cysteate- or sulfolactate-grown cells had major 42 kDa (and 8 kDa) protein bands. When the membrane fraction of the crude extract was separated from the soluble proteins by ultracentrifugation, this 42 kDa protein was found in the supernatant fluid, so it was presumed to be cytoplasmic.

Soluble proteins from the extracts of cysteate-grown cells were separated by anion-exchange chromatography (Fig. 4). Most of the protein eluted at about 0.3 M Na_2SO_4 , where cysteate aminotransferase, glutamate dehydrogenase, sulfite dehydrogenase and the 42 kDa and 8 kDa proteins were found. The highest peak was found to have the 42 kDa and 8 kDa proteins as its major components, and these presumptive subunits co-purified to near homogeneity (Fig. 3, lane 4) on hydrophobic interaction chromatography.

The purified protein was found to catalyse the sulfolactate sulfo-lyase reaction. When followed kinetically, pyruvate

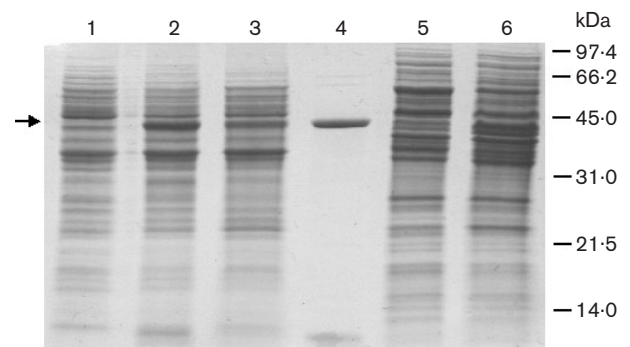


Fig. 3. Electropherogram of proteins in crude extracts of *P. pantotrophus* or *C. necator* grown under different conditions, and of the purified sulfolactate sulfo-lyase from *P. pantotrophus*. SDS-PAGE was done with crude extracts (30 μg protein) of *P. pantotrophus* grown with acetate (lane 1), cysteate (lane 2) and sulfolactate (lane 3), and of *C. necator* grown with acetate (lane 5) and sulfolactate (lane 6). Purified sulfolactate sulfo-lyase (5 μg) (lane 4) was initially recognized as a 42 kDa protein (arrow); the small subunit (8 kDa) was sometimes lost. The positions of the markers of molecular mass are indicated on the right.

was formed from sulfolactate at about 5 mkat ($\text{kg protein})^{-1}$. In discontinuous assays, disappearance of sulfolactate and formation of both pyruvate and sulfite were detected in amounts which confirmed the stoichiometry observed in Fig. 2.

The N-terminal amino acid sequence of the 42 kDa protein was determined to be LDFSNATVKAARREEGGVG. Three internal fragments were obtained: GNILGGLTTIEEK,

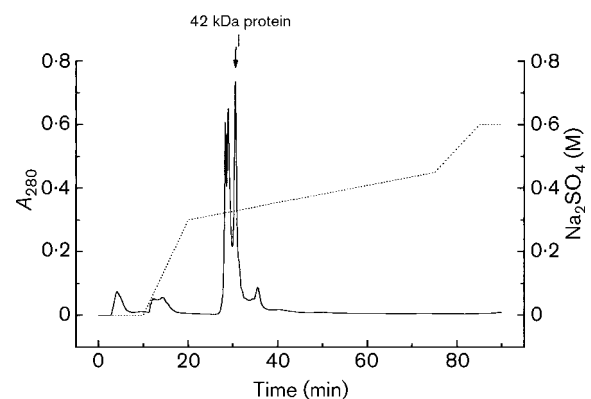


Fig. 4. Separation of the 42 kDa protein from extracts of cysteate-grown cells of *P. pantotrophus* on an anion-exchange column. Soluble proteins (5 mg) from cysteate-grown cells of strain NKNCYSA were applied to a 10 mm \times 10 cm Mono Q column in 20 mM Tris/sulfate buffer, pH 7.5. Elution of protein by a salt gradient was followed by monitoring A_{280} . The presence of the 42 kDa protein (with the 8 kDa protein) was monitored by SDS-PAGE (see Fig. 3).

ALGNLEK and GDFETIRQAGWK. The only one of these sequences to give similarities to a consistent set of gene products in a BLAST analysis was the first internal fragment, which had similarities to putative galactarate dehydratases (e.g. GarD, SSO1259; Table 1) of the correct length (about 390 amino acids). Galactarate dehydratase is part of the mandelate racemase group within the enolase superfamily (e.g. Babbitt *et al.*, 1996): the mechanism of this subgroup allowed the 'sulfolactate sulfo-lyase' (Suy) hypothesis in Fig. 1 to be generated.

Degenerate primers for PCR were derived from the peptides from the 42 kDa protein. The primer pair suy1-f and suy3-r yielded a 380 bp sequence, whose derived amino acid sequence overlapped with the sequences from the N-terminal peptide and the appropriate internal peptide. The sequence was thus not spurious. PCR primer pair suy2-f and suy4-r allowed the sequence to be extended to 780 bp, and primer walking with an adaptor-ligated gene bank (GenomeWalker kit) allowed the 5'- and the 3'-ends of the gene to be completed. The kit also allowed most of two upstream genes and a downstream gene to be sequenced. The small, upstream gene was presumed to encode the 8 kDa subunit of sulfolactate sulfo-lyase (Suy), and the genes were named *suyAB* (Fig. 1b).

The 8 kDa protein (SuyA) was encoded by a gene of 204 bp (67 amino acids, calc. 7327 Da). Comparison with the databases revealed a significant level of identity of position (39–51%) with short, (about 90 amino acids) putative proteins (Table 1), which were annotated as the N-terminus of altronate dehydratases. The 42 kDa protein (SuyB) was

encoded by a gene of 1182 bp (393 amino acids, calc. 42 451 Da). Comparison with the databases revealed (a) a high level of identity of position (31–66%) with putative proteins (Table 1) which had the correct length (about 390 amino acids) and which were often annotated as 'altronate dehydratases (C-terminal portion)', and (b) significant identity of position with the C-terminal region of characterized altronate and galactarate dehydratases (about 500 amino acids). In each case, the *suyAB*-like genes were contiguous (Table 1). Identities of up to 79% with SuyB were detected in environmental sequences (Table 1). It is apparent that *suyAB*-like genes are widespread in bacteria and in archaea, as well as in environmental samples (Table 1).

Many of the *SuyAB*-like sequences from Table 1 were compared with sequences of members of the enolase superfamily and with altronate dehydratases in a dendrogram (Fig. 5a). The sequences from seven isolated organisms grouped with *SuyAB* from *P. pantotrophus* strain NKNCYSA, and separate from representative altronate and galactarate dehydratases (UxaA and GarD), with some other *SuyAB*-like sequences (e.g. *N. aromaticivorans*).

A sulfate exporter?

The gene (1047 bp) downstream of *suyAB* could encode a product of 348 amino acids (calc. 36 061 Da) with high levels of identity of position (65–74%) with putative membrane proteins of unknown function (COG2855, e.g. TauZ in *Paracoccus denitrificans* NKNIS; Fig. 5b): none of these proteins was represented in the Transport

Table 1. Hypothetical *SuyAB*-like proteins deduced from sequences in different genomes and environmental samples, and growth with sulfolactate

Organism	Gene	Protein (amino acids)	Identity with SuyA (%)	Gene	Protein (amino acids)	Identity with SuyB (%)	Growth with sulfolactate
<i>P. pantotrophus</i> *	<i>suyA</i>	67	100	<i>suyB</i>	393	100	+
<i>Rubrobacter xylanophilus</i> *	Rxyl022789	92	39	Rxyl022790	387	66	ND
<i>Cupriavidus necator</i> *	Reut021543†	93	47	Reut021544	391	64	+
<i>Desulfotobacterium hafniense</i> *	Desu3012†	96	51	Desu3013	412	57	+
<i>Sulfolobus solfataricus</i>	SSO1260	99	49	SSO1259	387	52	ND
<i>Sulfolobus tokodaii</i>	ST2559	113	49	ST2560	387	52	ND
<i>Thermoplasma acidophilum</i>	Ta1392	95	45	Ta1393	387	52	ND
<i>Salmonella typhimurium</i> *	STM0649	113	37	STM0650	390	38	ND
<i>Campylobacter jejuni</i> *	Cj0482	87	46	Cj0483	390	35	ND
<i>Novosphingobium aromaticivorans</i>	Saro1433	123	41	Saro1434	402	31	–
Unknown 1‡	EAJ58423	96	66	EAJ58422	383	79	ND
Unknown 2*‡	EAJ27896	94	47	EAJ27897	382	70	ND
Unknown 3*‡	EAJ99662	43§	37	EAJ99663	388	68	ND

ND, Not done.

*ORF for hypothetical (sulfate) transporter nearby.

†Several candidates available.

‡Data from Venter *et al.*, 2004.

§Incomplete.

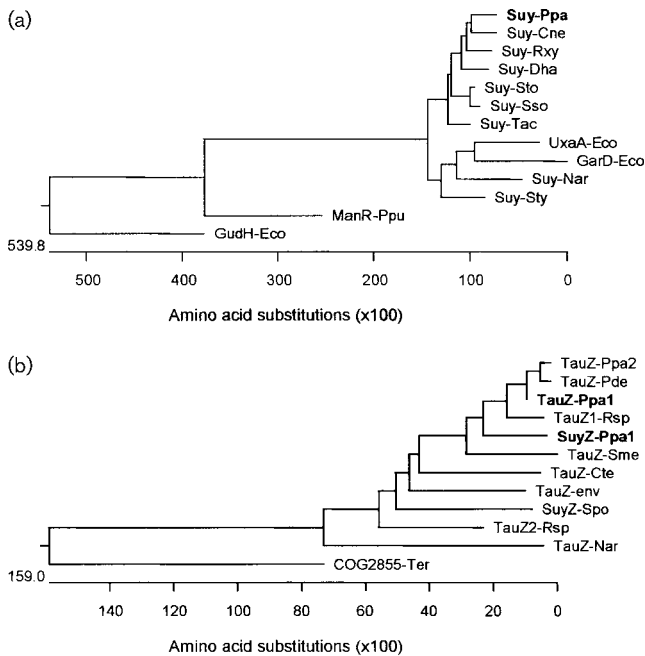


Fig. 5. Dendrograms, generated with CLUSTALW, of several dehydratases and SuyAB-like hypothetical proteins (a), and of SuyZ-like hypothetical proteins (b). The N-terminal sequences of the altronate and galactarate dehydratases correspond to SuyA, so the comparison was generated by considering SuyAB (abbreviated Suy) to be one protein. The abbreviations of the organisms [and the accession number of the relevant sequence(s)] are: Cne, *Cupriavidus necator* (ZP_00170328, ZP_00170329); Cte, *Chlorobium tepidum* (NP_661739); Dha, *Desulfitobacterium hafniense* (ZP_00099861, ZP_00099862); Eco, *Escherichia coli*; env, environmental sequence (EAF78647); Nar, *Novosphingobium aromaticivorans* (ZP_00302641); Pde, *Paracoccus denitrificans* (AAS78783); Ppa, *Paracoccus pantotrophus* [this paper (Ppa1), AAS78796 (Ppa2; DSM 65)]; Ppu, *Pseudomonas putida*; Rsp, *Rhodobacter sphaeroides* [ZP_00006687 (Rsp1), ZP_00005342 (Rsp2)]; Rxy, *Rubrobacter xylanophilus* (ZP_00186013); Sme, *Sinorhizobium melliloti* (NP_437511); Spo, *Silicibacter pomeroyi* (contig 2664, 172877–173860); Sso, *Sulfolobus solfataricus* (NP_342707, NP_342708); Sto, *Sulfolobus tokodaii* (NP_378561, NP_378562); Sty, *Salmonella typhimurium* (NP_459641, NP_459642); Tac, *Thermoplasma acidophilus* (NP_394846, NP_394847); Ter, *Trichodesmium erythraeum* (ZP_00328268). The sequences of four characterized enzymes are used: GarD, galactarate dehydratase (EC 4.2.1.42) (P39829); GudH, glucarate dehydratase (EC 4.2.1.40) (P76637); ManR, mandelate racemase (EC 5.1.2.2) (P11444); UxaA, altronate dehydratase (EC 4.2.1.7) (P42604).

Classification Database (June 2004; now TC 9.B.63.–.–). The gene was named *suyZ*, in analogy to *tauZ*. A *tauZ* gene was suspected in strain NKNCYSA (Brüggemann *et al.*, 2004), and it was found in the present study in the gene cluster encoding taurine dissimilation, where it is situated, as predicted, downstream of the gene (*xsc*) encoding the

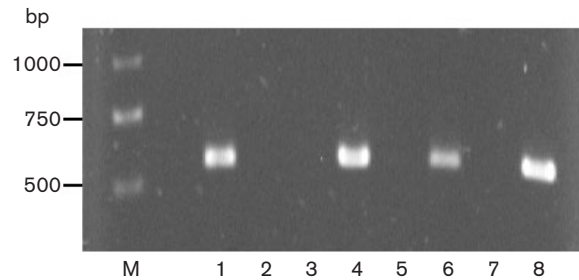


Fig. 6. Electropherogram of DNA fragments from RT-PCR to detect transcription of *suyZ* and *tauZ* genes. *P. pantotrophus* NKNCYSA was grown to mid-exponential phase in cysteate-, taurine- or acetate-salts medium. mRNA was separated from the harvested cells and subjected to RT-PCR. Lanes 1, 2 and 3, RT-PCR for transcript from *suyZ* with total RNA from cysteate-, taurine- and acetate-grown cells, respectively; lane 4, control PCR reaction with whole cells with the primer pair for *suyZ*; lanes 5, 6 and 7, RT-PCR for transcript from *tauZ* with total RNA from cysteate-, taurine- and acetate-grown cells, respectively; lane 8, control PCR reaction with whole cells with the primer pair for *tauZ*. Lane M, size markers.

desulfonative enzyme (GenBank accession no. AY498615). The two alleles, *suyZ* and *tauZ*, shared 73% identity of position over the sequenced portion of *tauZ*. The *suyZ*-like genes encode proteins (Fig. 5b), all of which are predicted to encode up to 10 transmembrane helices (THMM program). It was inferred that SuyZ (or TauZ) could be a sulfate exporter (Fig. 1a; see also below), so transcription was examined by RT-PCR.

It was possible to derive PCR primer pairs which distinguished between the transcripts from *suyZ* and from *tauZ*. Transcript from *suyZ* (primer pair *suyZ*-f and *suyZ*-r, product 548 bp) was detected only during growth with cysteate: no transcript was detected in cells growing with acetate or taurine (Fig. 6, lanes 1–3). Conversely, transcript from *tauZ* (primer pair *tauZ*-f and *tauZ*-r, product 539 bp) was obtained only during growth with taurine: no transcript was detected in cells growing with acetate or cysteate (Fig. 6, lanes 5–7). PCR reactions with whole cells as template for these primer pairs generated products of the correct size (Fig. 6, lanes 4, 8). Co-transcription of *suyB* and *suyZ* was then examined (not shown). A PCR reaction with whole cells as template for a forward primer in *suyB* (*suyB*-f) and a reverse primer in *suyZ* (*suyZ*-r) generated the anticipated fragment of 1224 bp. The same fragment was obtained in a RT-PCR with mRNA from cysteate-grown cells. There was no fragment in samples worked up from acetate-grown or taurine-grown cells. No fragment was obtained from the preparations of mRNA alone, so there was no contamination with DNA in the mRNA preparation. These results confirm the inducible transcription of the *suyZ* gene (Fig. 6, lanes 1–4), and show that the *suyBZ* genes are transcribed on a single mRNA molecule. It is inferred that the *suyABZ* genes represent (part of) an operon.

A regulator gene

An incomplete open reading frame (*orfR*) was found upstream of *suyA* (Fig. 1b). An NCBI Conserved Domain Search indicated some similarity to several potential transcriptional regulators, but no identity of position with a specific regulator was >38%. Whereas *suyAB*-like genes were found to be widespread (Table 1), there was no correlation between the occurrence of *orfR*-like genes and *suyAB*-like genes, so it seems unlikely that the regulator plays a role in sulfolactate metabolism.

Two pathways for cysteate dissimilation in prokaryotes?

Genes resembling *suyAB* were detected in the genomes of a wide range of prokaryotes (Table 1, Fig. 5a), many of which were impractical for us to examine, because they are thermophiles or pathogens. The candidates for *suyAB* in *Cupriavidus necator* JMP134, however, could be tested. The organism did not dissimilate cysteate, but it did dissimilate sulfolactate with quantitative substrate utilization and release of stoichiometric amounts of sulfate; inducible formation of a 42 kDa protein was detected by SDS-PAGE in cell extracts (Fig. 3, lanes 5–6). *Desulfotobacterium hafniense* also grew with sulfolactate, as implied by the presence of *suyAB*-like genes (Table 1) but not with cysteate. *Novosphingobium aromaticivorans*, whose *suyAB*-like genes shared the least identity with those in strain NKNCYSA (Table 1, Fig. 5a), grew with neither cysteate nor sulfolactate, so not all *suyAB*-candidates encode sulfolactate sulfo-lyase.

Bilophila wadsworthia RZATAU and *Desulfovibrio* sp. strain RZACYSA respire with cysteate (presumably as sulfite) as a sole electron acceptor to yield sulfide (Laue *et al.*, 1997a), and *Desulfovibrio* sp. strain GRZCYSA ferments cysteate to sulfate and sulfide (Laue *et al.*, 1997b). None of those organisms contained an inducible 42 kDa (or 8 kDa) protein, but each synthesized a cysteate-induced 35 kDa protein (not shown), as does *Desulfovibrio desulfuricans* IC1 (Lie *et al.*, 1998). In addition, each organism tested contained a cysteate transaminase. *Silicibacter pomeroyi* DSS-3^T is reported to dissimilate cysteate (González *et al.*, 2003), but the genome contains no *suyAB*-like genes. *Methanocaldococcus jannaschii* DSM 2661 utilizes cysteine-sulfinate, via sulfofpyruvate, as a sole source of nitrogen for growth with the release of sulfite (R. H. White, personal communication) but the genome does not contain the *suyAB* genes. It is inferred that at least one other pathway for the dissimilation of cysteate must exist.

DISCUSSION

The first catabolic reaction in the cysteate dissimilatory pathway of *P. pantotrophus* NKNCYSA is the constitutive transamination to 3-sulfofpyruvate (Fig. 1), coupled to regeneration of 2-oxoglutarate (Fig. 1). It is unclear whether a unique cysteate:2-oxoglutarate aminotransferase (EC 2.6.1.–) is expressed, or whether the reaction is catalysed by a protein with another function (e.g. aspartate transaminase, EC 2.6.1.1) (see for example Weinstein & Griffith, 1988). The second reaction in the pathway is an NAD-dependent reduction of 3-sulfofpyruvate to 3-sulfolactate (Fig. 1). Analogous to the first reaction, the enzyme is constitutive, and it is unclear whether a unique 3-sulfolactate dehydrogenase or a malate dehydrogenase (see Introduction) is involved. The specific activities of these enzymes were sufficient to explain the observed growth rate (cf. Mikosch *et al.*, 1999).

The crux of the pathway is the desulfonation reaction, sulfolactate sulfo-lyase (SuyAB; Fig. 1). It is clearly inducible (Fig. 3), and, with the generation of pyruvate, it releases the carbon moiety into amphibolic pathways.

The hypothesis that led us to this reaction and the structure of the pathway derived from the initial sequence data of the strongly induced 42 kDa protein (Fig. 3, lanes 1, 2 and 4), which contrasted with the constitutive initial enzymes in the pathway. The sequence data suggested an enzyme (galactarate dehydratase, GarD) in the mandelate racemase group of the enolase superfamily (e.g. Babbitt *et al.*, 1996): these enzymes have a substrate with a proton and a hydroxyl group on the α -carbon of a carboxylic acid. 3-Sulfolactate (Fig. 1) was thus the putative substrate. The mechanism of GarD (Gulick *et al.*, 1998) proceeds from the generation of a stabilized enolate (Fig. 7 shows the adaptation to cysteate) with a $\Delta 1$ double bond, to migration of the double bond to the $\Delta 2$ position. This migration in cysteate, with its sulfonate substituent on C3, would result in elimination of the good leaving group, sulfite, and the generation of an organic moiety (Fig. 7), which would tautomerize to pyruvate. We observed sulfite and pyruvate as the products (Fig. 2), so we presume that the postulated reaction mechanism has some validity. However, as more sequence data became available, the similarity to GarD was superseded by similarity to UxaA (Fig. 5a), an altronate dehydratase representing enzymes which often require Fe^{2+} for activity (Schomburg *et al.*, 2002). The enzyme assay was then reconstituted to accommodate iron. The phosphate buffer (pH 7.5) was replaced by MOPS (pH 6.5), and 1 mM Fe^{2+} was present. This was the first time that the

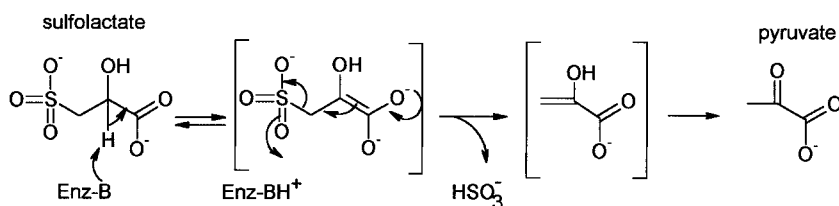


Fig. 7. Putative reaction mechanism of the sulfolactate sulfo-lyase. The relevant literature is cited in the text.

enzyme could be assayed. Further analysis showed that the iron was tightly bound to the enzyme, so the key to being able to assay the enzyme was not the presence of iron, but the correct choice of buffer. The tightly bound iron in SuyAB contrasts with the loosely bound iron (K_D about 4 mM) in the characterized altronate dehydratases (Dreyer, 1987).

Sulfolactate sulfo-lyase (SuyAB) is not represented in *Enzyme Nomenclature* (<http://www.chem.qmul.ac.uk/iubmb/enzyme/>). The desulfonation (Fig. 7), which is stoichiometric (Fig. 2) and is catalysed by a purified enzyme (see Fig. 3), apparently involves cleavage of a carbon–sulfur bond with formation (however transient) of a carbon–carbon double bond, and thus seems to fall within the carbon-sulfur lyases, EC 4.4.1.–. Sulfolactate sulfo-lyase seems to be an acceptable common name. A systematic name can be suggested when the absolute stereochemistry of the substrate has been established.

C. necator and *D. hafniense*, whose genomes contain *suyAB*-like genes, were found to utilize sulfolactate, so we presume that the cluster of proteins (Fig. 5a) with high similarity to SuyAB from strain NKNCYSA all encode sulfolactate sulfo-lyases, and that they are all members of the altronate dehydratase family. There are probably other members of this family whose reactions are unknown, namely the hypothetical proteins encoded by the *suyAB*-like genes in *N. aromaticivorans* (Table 1, Fig. 5a), and possibly those in *Salmonella typhimurium* (Table 1, Fig. 5a) and *Campylobacter jejuni* (Table 1).

Sulfolactate is a component of bacterial spores, so widespread degradative enzymes for this compound are not unexpected. A more recently recognized source of sulfolactate is sulfoquinovose, which disappears during growth, but whose desulfonation is incomplete (Roy *et al.*, 2003). One excretion product is sulfolactate; much more 3-sulfo-3-propane-1,2-diol is excreted. One could hypothesize that the substrate for desulfonation in these organisms is 3-sulfolactate, but that the enzyme loses activity, and that the better excretion form for the organisms to maintain homeostasis is the sulfo-3-propanediol. Cell suspensions of *P. pantotrophus* can rapidly lose the ability to desulfonate (see Results). There is no need to concentrate on sulfolactate as the substrate for desulfonation, because at least one other degradative pathway for cysteate exists, so a different compound could well be desulfonated.

Sulfite dehydrogenase (Fig. 1) is inducible as well, but as it is also induced during growth with taurine, it is regulated separately from the *suy* genes. Little is known about bacterial sulfite dehydrogenases (Kappler & Dahl, 2001) or their regulation. In the context of a catabolic pathway, the product of the reaction must be excreted to maintain a constant osmotic pressure in the cell (Fig. 1). Until now, it has been unclear what entity or system fulfilled this function (e.g. Brüggemann *et al.*, 2004).

The cytoplasmic membrane is impermeable to simple organosulfonate compounds (Graham *et al.*, 2002), so a transport system is essential for cysteate to enter *P. pantotrophus* NKNCYSA during growth (Fig. 1). Its nature is still unknown. The cell membrane is also impermeable to sulfate, whose transport into the cell has been studied for a generation (e.g. Pardee *et al.*, 1966) [TC 2.A.53.–]. The problem of exporting sulfate generated during dissimilatory reactions has received little attention (Brüggemann *et al.*, 2004; see also e.g. Homolya *et al.*, 2003). Indeed, Brüggemann *et al.* (2004) apparently had the answer in their gene cluster(s), because they recognized the need for an exporter, but they did not connect it with the ‘domain of unknown function’ attributed to the *tauZ* gene. It took another project, where the generation of sulfoacetate (from taurine) and the exporter of sulfoacetate are purportedly encoded by neighbouring genes (Denger *et al.*, 2004), to awaken the realization of the potential function of TauZ. Further, the mRNA for *suyZ* is part of a polycistronic message, which includes *suyB* (Fig. 6), so the gene product, which, as a member of COG2855, is membrane bound, presumably plays an equally important part in the degradative process. Osmotic homeostasis is a major candidate role for this protein, a suggestion which is strengthened by the fact that the corresponding protein, TauZ, is expressed in taurine-utilizing cells.

A transporter requires multiple transmembrane helices. TauZ and SuyZ from strain NKNCYSA are predicted to contain up to 10 transmembrane helices, as do all 12 tested hypothetical proteins in COG2855, which are found in the α -Proteobacteria and the Chlorobiaceae (Fig. 5b), as well as in (not shown) the β -Proteobacteria (48% identity of position), the γ -Proteobacteria (30%) and the Clostridiaceae (26%). The uppermost six TauZ/SuyZ proteins in Fig. 5(b) are all involved in established taurine or sulfolactate pathways. The data seem adequate to hypothesize that *suyZ* encodes a representative sulfate exporter. The relatively simple structure of the prediction, a single gene product with transmembrane helices but without any similarity to genes in the Transport Classification Database, leads us to the suggestion that SuyZ may represent a member of the poorly understood ion channels (see Kung & Blount, 2004).

Other phylogenetic groups of bacteria must also have sulfate exporters (ion channels), which have not been recognized, but which are necessary every time organisms require to excrete sulfate during the dissimilation of organosulfonates or sulfate esters. A re-examination of sequences in both real (*Burkholderia xenovorans* LB400) (Goris *et al.*, 2004) and hypothetical (*Leishmania major* clone P1023) β -Proteobacteria revealed *orfX* (COG0730) downstream of *pta*, in the degradative pathway of taurine (Ruff *et al.*, 2003). We were then able to predict and confirm the presence of an *orfX*-like gene downstream of *pta* in both *C. necator* JMP134 (Raet549701) and *Burkholderia* sp. strain R18194 (Bcepa02005097). It is thus inferred that COG0730 also represents a class of sulfate channels. Many

other exporters could exist in the many COG groups which encode putative membrane proteins with transmembrane helices, and dissimilatory pathways involving the excretion of phosphate, chloride or nitrite will presumably require them.

P. pantotrophus NKNCYSA is not the only organism in which two alleles of *suyZ* may be found. *Rhodobacter sphaeroides* (Fig. 5b) and *Sinorhizobium meliloti* (not shown) share this phenomenon. In each of the latter two organisms, one of the genes is *tauZ*, which is interpreted to encode the sulfate channel in taurine degradation; the pathway(s) for which the second candidate is required is unknown. *Silicibacter pomeroyi* has no *tauZ*-like gene adjacent to the other *tau* genes, where an alternative COG group can be postulated for the function. The *tauZ/suyZ*-like gene (Fig. 5b) may thus have a role in the unknown pathway for cysteate metabolism in this organism (see Results).

The short pathway of cysteate degradation (Fig. 1) seems to fall into three sections. Firstly, there is the unknown uptake system with the apparently constitutive expression of the steps releasing the ammonium ion and generating sulfolactate. Secondly there is the inducible sulfolactate sulfo-lyase, which is cotranscribed with the presumed sulfate export channel. And thirdly, there is the separately induced sulfite dehydrogenase. It seems possible that *P. pantotrophus* NKNCYSA is an organism that contains, in addition to the widespread regulated catabolism of sulfolactate and the separately regulated oxidation of sulfite, constitutive enzymes (possibly including transport) with other primary functions but a broad substrate range, that allow the degradation of cysteate to occur via sulfolactate.

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REFERENCES

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.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1987). *Current Protocols in Molecular Biology*. New York: Wiley.

Babbitt, P. C., Hasson, M. S., Wedekind, J. E. & 7 other authors (1996). The enolase superfamily: a general strategy for enzyme-catalyzed abstraction of the α -protons of carboxylic acids. *Biochemistry* **35**, 16489–16501.

Bonsen, P. P. M., Spudich, J. A., Nelson, D. L. & Kornberg, A. (1969). Biochemical studies of bacterial sporulation and germination. XII. A sulfonic acid as a major sulfur compound of *Bacillus subtilis* spores. *J Bacteriol* **98**, 62–68.

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.

Consdon, R., Gordon, A. H. & Martin, A. J. P. (1946). The identification of amino-acids derived from cysteine in chemically modified wool. *Biochem J* **40**, 580–582.

Cunningham, C., Tipton, K. F. & Dixon, H. B. F. (1998). Conversion of taurine into *N*-chlorotaurine (taurine chloramine) and sulphoacetaldehyde in response to oxidative stress. *Biochem J* **330**, 939–945.

Denger, K. & Cook, A. M. (2001). Ethanedisulfonate is degraded via sulfoacetaldehyde in *Ralstonia* sp. strain EDS1. *Arch Microbiol* **176**, 89–95.

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 structure. *Biochem J* **357**, 581–586.

Denger, K., Weinitschke, S., Hollemeyer, K. & Cook, A. M. (2004). Sulfoacetate generated by *Rhodospseudomonas palustris* from taurine. *Arch Microbiol* **182**, 254–258.

Dreyer, J. L. (1987). The role of iron in the activation of mannonic and altronic acid hydratases, two Fe-requiring hydro-lyases. *Eur J Biochem* **166**, 623–630.

González, J. M., Covert, J. S., Whitman, W. B. & 8 other authors (2003). *Silicibacter pomeroyi* sp. nov. and *Roseovarius nubinihibens* sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. *Int J Syst Evol Microbiol* **53**, 1261–1269.

Goris, J., Vos, P. D., Caballero-Mellado, J., Park, J., Falsen, E., Quensen, J. F. I., Tiedje, J. M. & Vandamme, P. (2004). Classification of the PCB- and biphenyl-degrading strain LB400 and relatives as *Burkholderia xenovorans* sp. nov. *Int J Syst Evol Microbiol* **54**, 1677–1681.

Graham, D. E. & White, R. H. (2002). Elucidation of methanogenic coenzyme biosyntheses: from spectroscopy to genomics. *Nat Prod Rep* **19**, 133–147.

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

Graupner, M., Xu, H. & White, R. H. (2000a). Identification of the gene encoding sulfo-pyruvate decarboxylase, an enzyme involved in biosynthesis of coenzyme M. *J Bacteriol* **182**, 4862–4867.

Graupner, M., Xu, H. & White, R. H. (2000b). Identification of an archaeal 2-hydroxy acid dehydrogenase catalyzing reactions involved in coenzyme biosynthesis in methanoarchaea. *J Bacteriol* **182**, 3688–3692.

- Gulick, A. M., Palmer, D. R., Babbitt, P. C., Gerlt, J. A. & Rayment, I. (1998).** Evolution of enzymatic activities in the enolase superfamily: crystal structure of (D)-glucarate dehydratase from *Pseudomonas putida*. *Biochemistry* **37**, 14358–14368.
- Homolya, L., Varadi, A. & Sarkadi, B. (2003).** Multidrug resistance-associated proteins: export pumps for conjugates with glutathione, glucuronate or sulfate. *Biofactors* **17**, 103–114.
- Kappler, U. & Dahl, C. (2001).** Enzymology and molecular biology of prokaryotic sulfite oxidation. *FEMS Microbiol Lett* **203**, 1–9.
- Kennedy, S. I. T. & Fewson, C. A. (1968).** Enzymes of the mandelate pathway in bacterium N.C.I.B. 8250. *Biochem J* **107**, 497–506.
- Kung, C. & Blount, P. (2004).** Channels in microbes: so many holes to fill. *Mol Microbiol* **53**, 373–380.
- Laemmli, U. K. (1970).** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Lamprecht, W. & Heinz, F. (1984).** Pyruvate. In *Methods of Enzymatic Analysis*, pp. 570–577. Edited by H. U. Bergmeyer. Weinheim: Verlag Chemie.
- Laue, H., Field, J. A. & Cook, A. M. (1996).** Bacterial desulfonation of the ethanesulfonate metabolite of the chloroacetanilide herbicide metazachlor. *Environ Sci Technol* **30**, 1129–1132.
- Laue, H., Denger, K. & Cook, A. M. (1997a).** Taurine reduction in anaerobic respiration of *Bilophila wadsworthia* RZATAU. *Appl Environ Microbiol* **63**, 2016–2021.
- Laue, H., Denger, K. & Cook, A. M. (1997b).** Fermentation of cysteate by a sulfate-reducing bacterium. *Arch Microbiol* **168**, 210–214.
- Lie, T. J., Pitta, T., Leadbetter, E. R., Godchaux, W., III & Leadbetter, J. R. (1996).** Sulfonates: novel electron acceptors in anaerobic respiration. *Arch Microbiol* **166**, 204–210.
- 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.
- Lie, T. J., Godchaux, W. & Leadbetter, E. R. (1999).** Sulfonates as terminal electron acceptors for growth of sulfite-reducing bacteria (*Desulfitobacterium* spp.) and sulfate-reducing bacteria: effects of inhibitors of sulfidogenesis. *Appl Environ Microbiol* **65**, 4611–4617.
- Mikosch, C., Denger, K., Schäfer, E.-M. & Cook, A. M. (1999).** Anaerobic oxidations of cysteate: degradation via a cysteate:2-oxoglutarate aminotransferase in *Paracoccus pantotrophus*. *Microbiology* **145**, 1153–1160.
- Pardee, A. B., Prestidge, L. S., Whipple, M. B. & Dreyfuss, J. (1966).** A binding site for sulfate and its relation to sulfate transport into *Salmonella typhimurium*. *J Biol Chem* **241**, 3962–3969.
- Reichenbecher, W., Kelly, D. P. & Murrell, J. C. (1999).** Desulfonation of propanesulfonic acid by *Comamonas acidovorans* strain P53: evidence for an alkanesulfonate sulfonate and an atypical sulfite dehydrogenase. *Arch Microbiol* **172**, 387–392.
- Roy, A. B., Hewlins, M. J. E., Ellis, A. J., Harwood, J. L. & White, G. F. (2003).** Glycolytic breakdown of sulfoquinovose in bacteria: a missing link in the sulfur cycle. *Appl Environ Microbiol* **69**, 6434–6441.
- 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.
- Sanger, F. (1945).** The free amino groups of insulin. *Biochem J* **39**, 507–515.
- Schläfli, H. R., Weiss, M. A., Leisinger, T. & Cook, A. M. (1994).** Terephthalate 1,2-dioxygenase system from *Comamonas testosteroni* T-2: purification and some properties of the oxygenase component. *J Bacteriol* **176**, 6644–6652.
- Schleheck, D., Dong, W., Denger, K., Heinzle, E. & Cook, A. M. (2000).** An α -proteobacterium converts linear alkylbenzenesulfonate (LAS) surfactants into sulfophenylcarboxylates, and linear alkyldiphenyletherdisulfonate surfactants into sulfodiphenylethercarboxylates. *Appl Environ Microbiol* **66**, 1911–1916.
- Schmidt, E. (1974).** Glutamat-dehydrogenase UV-test. In *Methoden der Enzymatischen Analyse*, pp. 689–696. Edited by H. U. Bergmeyer. Weinheim: Verlag Chemie.
- Schomburg, D., Schomburg, I. & Chang, A. (2002).** Lyases II: EC 4.1.3–4.2.1. In *Springer Handbook of Enzymes*. Edited by D. Schomburg & I. Schomburg. Berlin: Springer.
- Sörbo, B. (1987).** Sulfate: turbidimetric and nephelometric methods. *Methods Enzymol* **143**, 3–6.
- Stapley, E. O. & Starkey, R. L. (1970).** Decomposition of cysteic acid and taurine by soil microorganisms. *J Gen Microbiol* **64**, 77–84.
- Tholey, A., Wittmann, C., Kang, M. J., Bungert, D., Hollemeyer, K. & Heinzle, E. (2002).** Derivatization of small biomolecules for optimized matrix-assisted laser desorption/ionization mass spectrometry. *J Mass Spectrom* **37**, 963–973.
- Tralau, T., Cook, A. M. & Ruff, J. (2003a).** An additional regulator, TsaQ, is involved with TsaR in regulation of transport during the degradation of *p*-toluenesulfonate in *Comamonas testosteroni* T-2. *Arch Microbiol* **180**, 319–326.
- Tralau, T., Mampel, J., Cook, A. M. & Ruff, J. (2003b).** Characterization of TsaR, an oxygen-sensitive LysR-type regulator for the degradation of *p*-toluenesulfonate in *Comamonas testosteroni* T-2. *Appl Environ Microbiol* **69**, 2298–2305.
- Vandamme, P. & Coenye, T. (2004).** Taxonomy of the genus *Cupriavidus*: a tale of lost and found. *Int J Syst Evol Microbiol* **54**, 2285–2289.
- Venter, J. C., Remington, K., Heidelberg, J. F. & 20 other authors (2004).** Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304**, 66–74.
- 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.
- Weinstein, C. L. & Griffith, O. W. (1986).** β -Sulfoxyruvate: chemical and enzymatic syntheses and enzymatic assay. *Anal Biochem* **156**, 154–160.
- Weinstein, C. L. & Griffith, O. W. (1988).** Cysteinesulfonate and β -sulfoxyruvate metabolism. Partitioning between decarboxylation, transamination, and reduction pathways. *J Biol Chem* **263**, 3735–3743.
- White, R. H. (1984).** Biosynthesis of the sulfonolipid 2-amino-3-hydroxy-15-methylhexadecane-1-sulfonic acid in the gliding bacterium *Cytophaga johnsonae*. *J Bacteriol* **159**, 42–46.
- White, R. H. (1986).** Intermediates in the biosynthesis of coenzyme M (2-mercaptoethanesulfonic acid). *Biochemistry* **25**, 5304–5308.
- Widdel, F. & Pfennig, N. (1981).** Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. *Arch Microbiol* **129**, 395–400.
- Wood, D. A. (1971).** Sporulation in *Bacillus subtilis*. The appearance of sulpholactic acid as a marker event for sporulation. *Biochem J* **123**, 601–605.