Ethanedisulfonate is degraded via sulfoacetaldehyde in *Ralstonia* sp. strain EDS1

Abstract Aerobic enrichment cultures (11) yielded three cultures able to utilise ethane-1,2-disulfonate as sole source of carbon and energy in salts medium. Two pure cultures were obtained and we worked with strain EDS1, which was assigned to the genus *Ralstonia* on the basis of its 16S rDNA sequence and simple taxonomic tests. Strain EDS1 utilised at least seven alkane(di)sulfonates, ethane-1,2-disulfonate, taurine, isethionate, sulfoacetate, sulfoacetaldehyde and propane-1,3-disulfonate, as well as methane-sulfonate and formate. Growth with ethanedisulfonate was concomitant with substrate disappearance and the formation of 2 mol sulfate per mol substrate. The growth yield, 7 g protein (mol C)⁻¹, indicated quantitative utilisation of the substrate. Ethanedisulfonate-dependent oxygen uptake of whole cells during growth rose to a maximum before the end of growth and then sank rapidly; this was interpreted as evidence for an inducible desulfonative oxygenase that was not active in cell extracts. Inducible sulfoacetaldehyde sulfo-lyase was detected at high activity. Inducible degradation of taurine or isethionate or sulfoacetate via sulfoacetaldehyde sulfo-lyase is interpreted from the data.

Keywords Desulfonation · Disulfonates · Oxygenase · *Ralstonia* · Sulfoacetaldehyde sulfo-lyase

Introduction

An increasing range of sulfonated xenobiotic compounds is being disposed of safely in sewage treatment plants via degradation in activated sludge. Usually, only biodegradability has been established, as for primary alkanesulfonates, secondary alkanesulfonates and α -olefinsulfonates (Schöberl 1993), which are used as surfactants; primary

alkanesulfonates are also used as ion-pair reagents (e.g. Adams 1985). Recently, however, a vastly improved understanding of the degradation of alkanesulfonates has been generated, largely from work in pure culture with the natural product, methanesulfonate and its homologues (Baker et al. 1991; Kelly and Murrell 1999; Reichenbecher et al. 1999; Reichenbecher and Murrell 1999). The key reaction, desulfonation, is catalysed by a multi-component, mono-nuclear-iron monooxygenase (Reichenbecher and Murrell 2000).

Some commercial surfactants are derivatised natural products, e.g. taurine (2-aminoethanesulfonate) and isethionate (2-hydroxyethanesulfonate). The underivatised compounds are apparently degraded via sulfoacetaldehyde, which is hydrolysed by thiamine pyrophosphate (TPP)-coupled sulfoacetaldehyde sulfo-lyase to acetate and sulfite [E.C. 4.4.1.12] (Cook et al. 1999; Kondo and Ishimoto 1975).

A different source of xenobiotic sulfonates in sewage works is pharmaceuticals, some of whose widely used active ingredients (e.g. clomethiazole, prochlorperazine as sedatives) are formulated with ethane-1,2-disulfonate (Fig. 1) as the counter-ion (Budavari 1989). Little seems to be known about this class of compounds; butane-1,4-disulfonate was not degraded in standard tests (OECD 301E and 302B cf. ref. OECD 1992) (Sicherheitsdatenblatt, Knoll Co., Ludwigshafen).

We now report on the easy enrichment of cultures of aerobic bacteria able to degrade ethanedisulfonate as the sole source of carbon and energy for growth. One isolate, assigned to the genus *Ralstonia*, mineralises ethanedisulfonate via oxygenation and the sulfoacetaldehyde sulfo-lyase reaction.

Materials and methods

Inocula, growth medium, enrichment cultures, and isolation and identification of bacteria

Inocula for enrichment cultures were from pristine lake sediment (Alastradero Lake, California, USA), garden soil (Konstanz, Germany), communal activated sludge (Corvallis, Ore., USA, and

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Fig. 1 Ethane-1,2-disulfonate (EDS), some naturally occurring organosulfonates, and the probable role of sulfoacetaldehyde sulfo-lyase [E.C. 4.4.1.12] in their degradation in *Ralstonia* sp. strain EDS1. The lines in **bold** indicate reactions for which direct or indirect evidence is provided. *TAU* Taurine, *ISE* isethionate, *SAA* sulfoacetaldehyde, *SAC* sulfoacetate, *AC* acetate

Herisau, Switzerland), industrial activated sludge (Ludwigshafen, Germany), anaerobically digested sludge (Corvallis, Ore., USA, and Pushchino, Russia), anoxic sediment (Lake Constance, Germany) and faecal material from cow, chicken and mice.

A phosphate-buffered minimal-salts medium, pH 7.2 (Thurnheer et al. 1986), was used throughout the work. Aerobic enrichment cultures were prepared in salts medium containing 10 mM ethane-1,2-disulfonate as the sole source of carbon and energy. Enrichment cultures (5 ml in 30-ml screw-cap tubes) were set up under aseptic conditions with a single inoculum, and incubated in the dark at 30 °C on a roller. Cultures were considered positive after five passages in enrichment medium and they were then streaked on LB-agar plates. Colonies were picked to 10 mM ethanedisulfonate-salts medium and a culture was considered pure after three rounds of streaking and picking yielded homogeneity on plates and under the microscope. Short-term storage was in liquid medium at 4 °C.

Standard methods were used to establish Gram reaction, catalase and cytochrome *c* oxidase activity (Gerhardt et al. 1994). Motility and morphology were examined microscopically. Strain EDS1 was subject to partial sequencing (~ 450 nucleotides) of the 16S rDNA (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; DSMZ), and the sequence was aligned and evaluated by DSMZ as described elsewhere (Maidak et al. 1996; Rainey et al. 1996). Strain EDS1 was deposited with the DSMZ as DSM 13640.

Growth physiology, cell suspension experiments and enzyme assays

Substrate utilisation was usually evaluated in duplicate 5-ml cultures with 10 mM organic carbon (20 mM methanesulfonate or formate, 5 mM glucose, benzoates or methanol) in 30-ml air-tight tubes. End-point determinations were made. Each growth experi-

ment was done in a 100-ml culture with 10 mM ethanedisulfonate-salts medium in a 500-ml Erlenmeyer flask on a rotary shaker at 30 °C. Samples were taken at intervals to measure the OD_{580} and to determine the concentration of protein, ethanedisulfonate and sulfate. When oxygen uptake in whole cells was followed during growth, the initial culture volume was 250 ml in a 1-l Erlenmeyer flask shaken at 30 °C. Portions of the culture were harvested by centrifugation (20,000×g, 5 min, 20 °C) and the cells washed once with 50 mM Tris-HCl, pH 7.5. Washed cells were immediately resuspended in the Tris buffer to 0.03–2.0 mg protein ml^{-1} and the ethanedisulfonate-dependent oxygen uptake was determined in a Clarke-type electrode; sulfonate was present at 2 mM. When large amounts of cells were required for use in cell suspensions or cell-free work, several 100-ml cultures were prepared and the cells were harvested (20,000×g, 5 min, 4 °C) when the specific oxygen uptake was maximal (see below). Cells were washed in cold 0.1 M potassium phosphate buffer, pH 7.5, which contained 5 mM MgCl_2 , and immediately disrupted by three passages through a chilled French pressure cell set at 140 MPa. Whole cells and debris were removed by centrifugation (20,000×g, 5 min, 4 °C) and the supernatant fluid was used for experiments. Analyses for oxygenases were done immediately; samples for the analysis of sulfoacetaldehyde sulfo-lyase could be stored for several weeks at –20 °C. We consider protein to be about 50% of the cell dry weight (Fuchs 1999).

Sulfoacetaldehyde sulfo-lyase [E.C. 4.4.1.12] was assayed in a discontinuous test (Denger et al. 2001; King et al. 1997). The reaction mixture (1 ml) contained 100 μmol potassium phosphate, pH 7.5, 5 μmol MgCl_2 , 10 μmol sulfoacetaldehyde, 1 μmol TPP, and 0.5–0.9 mg of protein, with which the assay was started at 30 °C. Samples were routinely taken at intervals into formic acid and the formation of acetate was determined. Some reactions were followed as substrate disappearance or formation of sulfite.

Iso-citrate lyase [E.C. 4.1.3.1] was assayed as release of glyoxylate in a colorimetric assay (Dixon and Kornberg 1959). Sulfite

dehydrogenase [E.C. 1.8.99.-] was assayed as reduction of ferricyanide (Reichenbecher et al. 1999).

Putative ethanedisonate monooxygenase in crude extracts was assayed as substrate-dependent uptake of oxygen in the oxygen electrode, as a decrease of ethanedisonate or as an increase in sulfate by ion chromatography, or as appearance of sulfoacetaldehyde by HPLC. The reaction mixture in the oxygen electrode (0.5 ml) at 30 °C contained 25 µmol Tris-HCl, pH 7.5, 20 nmol FeCl₃ or 100 nmol FeSO₄, 200 nmol NADH or NADPH, 0.5–3.5 mg of protein, and 10 µmol ethanedisonate, with which the reaction was started. In other assays, 100 nmol FAD, 200 nmol FMN, 200 nmol ascorbate or 500 nmol 2-oxoglutarate was added in addition to the NAD(P)H.

Analytical methods

Ethanedisonate, sulfoacetate and sulfate were routinely determined by ion chromatography with suppression and conductivity detection (Laue et al. 1996). Sulfate was occasionally assayed as the suspended barium salt (Sörbo 1987). Sulfite was routinely determined as the fuchsin complex (Kondo et al. 1982) and occasionally examined by ion chromatography (Laue et al. 1996). Acetate was determined as acetic acid by gas chromatography (Laue et al. 1997). Sulfoacetaldehyde (Cunningham et al. 1998) and taurine (Denger et al. 1997) were determined by HPLC after derivatisation. SDS-PAGE was done with 13% separative gels (Laemmli 1970); low-range marker proteins (Bio-Rad) were used. Protein was assayed by a Lowry-type method (Kennedy and Fewson 1968).

Materials

Ethane-1,2-disulfonate was from Sigma. Sulfoacetaldehyde was synthesised as the bisulfite addition complex (Denger et al. 2001). Other chemicals were of the highest purity available, from Fluka, Sigma-Aldrich or Merck.

Results

Enrichment cultures for the degradation of ethanedisonate were set up with 11 inocula and three were positive, those with inocula of activated sludge from two communal treatment plants and from garden soil. Cultures grew within 3 days after the first transfer. The inocula from largely anoxic environments (6), industrial activated sludge (1) and pristine lake sediment (1) gave no enrichment 10 days after transfer. The three enrichment cultures were microscopically indistinguishable, two of the enrichments were worked up to yield pure cultures able to utilise ethanedisonate, strains EDS1 (from activated sludge in Corvallis) and EDS2 (from soil). Strains EDS1 and EDS2 were short (0.6 to 1.0 µm×0.5 µm), motile, gram-negative rods that were oxidase- and catalase-positive. The partial 16S rDNA sequence of strain EDS1 showed highest similarity to the *Ralstonia*-group (Coenye et al. 1999) of the β-Proteobacteria (*R. pickettii*^T, 95.1%; *R. solanacearum*^T, 95.1%; *R. eutropha*^T, 94.8%; *R. paucula*^T, 93.1%; *R. gilardii*^T, 92.1%), which is compatible with the simple taxonomy done above. Strain EDS1 did not grow with glucose or protocatechuate, and it did not reduce nitrate, so it probably cannot be assigned to either *R. pickettii* or *R. solanacearum*. We suspect strain EDS1 to be a novel species of the genus *Ralstonia*.

Fig. 2A–C Growth of *Ralstonia* sp. strain EDS1 in 10 mM ethanedisonate-salts medium with substrate utilisation, product formation and the specific activity of ethanedisonate oxygenation in whole cells. Protein (■) concentration during growth (A), ethanedisonate (Δ) and sulfate (●) concentrations as a function of growth (B) and the specific activity of ethanedisonate-dependent oxygen uptake (○) by whole cells during growth (C)

Ralstonia sp. strain EDS1 grew with ethanedisonate, taurine, isethionate, sulfoacetaldehyde, sulfoacetate, methanesulfonate and propane-1,3-disulfonate, but not with ethanesulfonate, propanesulfonate, pentanesulfonate, heptanesulfonate, cysteine, coenzyme M or *p*-toluenesulfonate. Non-sulfonated growth substrates included formate, acetate, propionate and succinate but not methanol, glycolate, glyoxylate, benzoate or 4-hydroxybenzoate.

Table 1 Quantitative aspects of growth of *Ralstonia* sp. strain EDS1 with different substrates

Growth substrate	Sulfate formed ^b mol (mol substrate) ⁻¹	Molar growth yield g protein (mol C) ⁻¹
Acetate ^a	0	6.8
Ethane-1,2-disulfonate ^a	2.0	6.7
Taurine ^a	1.2	6.6
Isethionate	1.4	7.4
Sulfoacetate ^a	1.1	4.4
Sulfoacetaldehyde ^a	1.0	7.9
Propane-1,3-disulfonate	2.0	6.8
Methanesulfonate	1.2	7.6
Formate	0	6.6

^aThis compound could be determined in growth medium and was utilized quantitatively

^bWhere the substrate could not be determined, the nominal concentration was used for calculations. The values indicate that each substrate was utilised quantitatively

Strain EDS1 grew exponentially with ethanedisulfonate ($\mu=0.07\text{ h}^{-1}$) concomitant with substrate utilisation and excretion of sulfate (Fig. 2A, B), which was identified by co-chromatography with authentic material on ion chromatography and its ability to precipitate Ba^{2+} . The yield of sulfate (Fig. 2B), 2.0 mol (mol ethanedisulfonate)⁻¹, corresponded with the theoretical value for the complete oxidation of ethanedisulfonate. The molar growth yield, 7 g protein (mol C)⁻¹, also corresponded to complete oxidation of the substrate with incorporation of about 50% of the carbon into biomass (e.g. Cook 1987). The data from a corresponding experiment with strain EDS2 were indistinguishable from those shown in Fig. 2 (not shown).

Growth with all sulfonated compounds led to the quantitative release of the sulfonate group(s) as sulfate (Table 1). Intermittent determinations for sulfite from many cultures were all negative. Growth with acetate, during which no release of sulfate was detected (Table 2), confirmed that the determinations of sulfate were not spurious. Most growth yields were about 7–8 g protein (mol C)⁻¹; only sulfoacetate was much lower (4.4 g protein (mol C)⁻¹; Table 1).

The specific activity of ethanedisulfonate turnover by whole cells of strain EDS1, measured as uptake of oxy-

gen, varied markedly during growth (Fig. 2C). At low cell densities, no activity was observed. The specific activity then rose sharply to a maximum of about 5.2 mkat (kg protein)⁻¹ some 5 h before the end of growth, and then fell equally rapidly to undetectable levels. This behaviour is reminiscent of that of many multi-component oxygenases during growth (e.g. Junker et al. 1994; Mampel et al. 1999; Moodie et al. 1990), so we presumed the initial attack on ethanedisulfonate to be by monooxygenation, analogous to the attack on methanesulfonate (e.g. Reichenbecher and Murrell 2000). Whole cells of strain EDS1 were able to desulfonate ethanedisulfonate only if molecular oxygen was available; no sulfite, only sulfate was detected as a product. There was no desulfonation in the absence of molecular oxygen. Despite experience with oxygenases (e.g. Cook et al. 1999; Reichenbecher and Murrell 2000), we were unable to detect turnover of ethanedisulfonate in cell extracts whether determined directly or indirectly, but we presumed that we had unsuitable conditions for an ethanedisulfonate monooxygenase and maintained the working hypothesis of an oxygenolytic desulfonation as the first catabolic step. This hypothesis indicated 2-sulfoacetaldehyde as the product of desulfonation and further metabolism via acetate (Fig. 1).

We detected the activity of sulfoacetaldehyde sulfo-lyase in crude extracts of strain EDS1. Acetate was detected and quantified by gas chromatography concomitant with the disappearance of sulfoacetaldehyde. Sulfite was quantified colourimetrically and its identity confirmed by ion chromatography.

Sulfite dehydrogenase activity was observed at high specific activity (2.7 mkat (kg protein)⁻¹) in extracts of cells grown with ethanedisulfonate, but at low levels in extracts of acetate-grown cells (0.2 mkat (kg protein)⁻¹). Isocitrate lyase, a marker enzyme for the glyoxylate cycle, was active in extracts of cells grown with ethanedisulfonate (0.5 mkat (kg protein)⁻¹) and sulfoacetate (0.3 mkat (kg protein)⁻¹), but was present in much lower amounts in cells grown with methanesulfonate (0.02 mkat (kg protein)⁻¹).

Cells grown with ethanedisulfonate displayed a high oxygen uptake with ethanedisulfonate (3.8 mkat (kg protein)⁻¹; Table 2), a significant oxygen uptake with acetate and a low oxygen uptake with taurine. The specific activity of sulfoacetaldehyde sulfo-lyase in cell extracts was

Table 2 Specific activities of oxygen uptake by whole cells and of sulfoacetaldehyde sulfo-lyase in extracts of *Ralstonia* sp. strain EDS1 after growth with different substrates. The substrates for whole-cell assays were present at 1.6–2.0 mM. EDS Ethane-1,2-disulfonate, AC acetate, TAU taurine, ISE isethionate, SAC sulfoacetate, MS methanesulfonate, PDS propane-1,3-disulfonate, NA not assayed

Growth substrate	Specific activity of oxygen uptake (mkat (kg protein) ⁻¹) in whole cells with							Sulfo-lyase mkat (kg protein) ⁻¹
	EDS	AC	TAU	ISE	SAC	MS	PDS	
Ethane-1,2-disulfonate	3.8	2.4	0.7	NA	NA	≤0.1	≤0.1	5.7
Acetate	0.3	1.4	≤0.1	NA	NA	NA	NA	≤0.1
Taurine	0.2	1.4	3.7	NA	NA	NA	NA	2.5
Isethionate	≤0.1	1.8	0.7	3.4	0.3	≤0.1	≤0.1	1.3
Sulfoacetate	≤0.1	1.5	0.4	0.4	1.3	≤0.1	≤0.1	1.5
Methanesulfonate	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	9.6	≤0.1	≤0.1
Propane-1,3-disulfonate	0.7	0.3	0.2	≤0.1	≤0.1	≤0.1	1.3	≤0.1

Fig. 3 Electropherogram of proteins in crude extracts of *Ralstonia* sp. strain EDS1 grown with different carbon sources. SDS-PAGE was done with extracts (30 μ g protein) of cells grown with ethanedisulfonate (lane 1), taurine (lane 2), isethionate (lane 3), sulfoacetate (lane 4), acetate (lane 5), methanesulfonate (lane 6) or propanedisulfonate (lane 7). The molecular masses refer to the marker proteins in the right-hand lane. The arrow indicates the molecular mass of sulfoacetaldehyde sulfo-lyase from *D. thiosulfatigenes* (Denger et al. 2001) and *Alcaligenes* sp. strain NKNTAU (Denger, unpublished data)

high (5.7 mkat (kg protein)⁻¹). Neither methanesulfonate nor propanedisulfonate caused oxygen uptake with these cells. Cells grown with acetate showed the highest oxygen uptake with acetate, low activity with ethanedisulfonate and none with taurine; the corresponding crude extracts had no detectable sulfoacetaldehyde sulfo-lyase. Taurine-grown cells had the highest specific activity with taurine, somewhat lower with acetate and negligible with ethanedisulfonate; a significant level of sulfoacetaldehyde sulfo-lyase was observed in cell extracts (Table 2). Isethionate-grown cells had the highest specific activity with isethionate, significant with acetate and low or negligible with other substrates; sulfoacetaldehyde sulfo-lyase was present in significant activities in cell extracts. Sulfoacetate-grown cells had equally high activities with sulfoacetate (1.3 mkat (kg protein)⁻¹) and acetate and cell extracts contained significant sulfoacetaldehyde sulfo-lyase activity (Table 2); whole cells showed little oxygen uptake with other compounds tested routinely (Table 2) or with glycollate or glyoxylate (not shown). Methanesulfonate-grown cells showed oxygen uptake with methanesulfonate, but no other activity. Propanedisulfonate-grown cells had significant oxygen uptake with propanedisulfonate, but, apart from ethanedisulfonate no other significant activity; possibly the putative propanedisulfonate-monooxygenase (and transport) could accept ethanedisulfonate as a substrate.

We postulated a common sulfoacetaldehyde sulfo-lyase for the catabolism of ethanedisulfonate, taurine, isethionate, sulfoacetate (Table 2) and sulfoacetaldehyde (Table 1), along with individually inducible enzymes to convert the appropriate growth substrate into sulfoacetaldehyde (Table 2). Cells grown with acetate, methanesulfonate and propanedisulfonate utilise other pathways (Table 2). We thus anticipated a protein band at about 64 kDa, representing the molecular mass of sulfoacetaldehyde sulfo-lyase found elsewhere in our work (Denger et al. 2001 and Denger, unpublished) and possibly in isethionate-

grown cells elsewhere (Lie et al. 1998). SDS-PAGE of the crude extracts used in Table 2 confirmed this idea. Cells grown with ethanedisulfonate, taurine, isethionate and sulfoacetate contained a common band at about 64 kDa; cells grown with acetate, methanesulfonate or propanedisulfonate did not (Fig. 3). Each extract had a slightly different pattern, in agreement with the idea of inducible enzymes for the different growth substrates (Fig. 3, see Table 2). Bands representing monomers of 46 kDa and 22 kDa were prominent in extracts from cells grown with ethanedisulfonate; similar bands (43 and 21 kDa) in methanesulfonate-grown cells and propanedisulfonate-grown cells (47 and 22 kDa) were observed (Fig. 3).

Discussion

There was no difficulty in obtaining organisms to utilise ethanedisulfonate as a source of carbon and energy for aerobic growth, though the aerated section of a communal sewage treatment plant seems to provide the best chance to obtain degradation of this component of pharmaceuticals that are disposed of in liquid wastes. The organisms we obtained were presumably all *Ralstonia* spp., which are common in sewage works and soil (Madigan et al. 2000). *Ralstonia* is a member of the β -Proteobacteria, so its ability to utilize a C-1 compound such as formate is not surprising (Madigan et al. 2000); the utilization of methanesulfonate, however, would appear to be the first example of this type of metabolism outside of the α -Proteobacteria (Kelly and Murrell 1999).

The specific degradation rate of strain EDS1 utilising ethanedisulfonate, calculated from the specific growth rate and the molar growth yield (ethanedisulfonate basis), is 1.5 mkat (kg protein)⁻¹. We presume there to be a transport system for this highly polar compound (see Cook et al. 1999). We also presume (Fig. 2C) that the first metabolic reaction is an oxygenation to yield sulfoacetalde-

hyde (Fig. 1), analogous to the oxygenation of methanesulfonate and longer-chain alkanesulfonates (Reichenbecher et al. 1999; Reichenbecher and Murrell 2000). We could not prove this, but the failure of whole cells to attack ethanedisulfonate until oxygen is introduced into the system lends support to the hypothesis, as do specific protein bands (Fig. 3), which could correspond to an oxygenase component similar to that in the methanesulfonate monooxygenase system (see Reichenbecher and Murrell 2000). The inducible enzymes causing oxygen uptake in the presence of ethanedisulfonate have a sufficient specific activity to explain the growth rate (Table 2), as does the activity of the sulfoacetaldehyde sulfo-lyase in these cells (Table 2). Acetate, the next intermediate, which is observed as the product of sulfoacetaldehyde sulfo-lyase, also causes significant oxygen uptake. There is thus both direct evidence for part of the suggested pathway and indirect support for the whole ethane-1,2-disulfonate degradation pathway shown in Fig. 1. We have thus observed the first known pathway for the degradation of a disulfonated aliphatic compound and we postulate that it contains two different mechanisms of desulfonation, i.e. oxygenation and hydrolysis. We detected a highly active, inducible sulfite dehydrogenase in cell extracts (see Johnston et al. 1975; Reichenbecher et al. 1999), and we presume that this enzyme activity explains why no sulfite is detected outside the cells during growth.

We presume that taurine is also degraded via sulfoacetaldehyde (Figs. 1, 3). The activity of sulfoacetaldehyde sulfo-lyase in taurine-grown cells is relatively high (Table 2) and the taurine-grown cells have a relatively high activity against acetate (Table 2). This pathway corresponds to that in *Achromobacter xylosoxidans* subsp. *denitrificans* NCIMB 10751 (Kondo and Ishimoto 1975), *Pseudomonas aeruginosa* (Shimamoto and Berk 1980), *Burkholderia cepacia* (King et al. 1997), and in anaerobically grown *Desulfonispora thiosulfatigenes* (Denger et al. 2001) and *Alcaligenes* sp. (Denger, unpublished data).

Analogous to the arguments with taurine, we anticipate isethionate to be metabolised via sulfoacetaldehyde (Table 2, Fig. 3), as indicated for an unidentified isolate (Kondo et al. 1977 and for *Acinetobacter* sp. (King et al. 1997).

The degradation of sulfoacetate via glycollate (Martelli and Sousa 1970) or sulfoacetaldehyde (King and Quinn 1997) has been proposed. Strain EDS1 does not grow with glycollate (or glyoxylate), and sulfoacetate-grown cells do not oxidise these compounds, which contrasts with the pathway via glycollate (Martelli and Sousa 1970). However, these cells oxidise sulfoacetate and acetate equally well, and they contain significant levels of sulfoacetaldehyde sulfo-lyase (Table 2; see Fig. 3). We thus believe that the pathway suggested by King and Quinn (1997) is present in strain EDS1, especially as the inducible marker enzyme for the glyoxylate pathway, isocitrate lyase, is present during growth with sulfoacetate (and ethanedisulfonate) and effectively absent in methanesulfonate-grown cells. The oddity of the pathway (Fig. 1) is the reduction of the carboxylic acid to the aldehyde.

The reaction type is known in aerobes in biosynthetic reactions such as the first steps in proline biosynthesis, glutamate 5-kinase [E.C. 2.7.2.11] and L-glutamate 5-semialdehyde dehydrogenase [E.C. 1.2.1.41]. This pair of reactions to reduce a carboxylate costs one ATP and one NADPH. So instead of the oxidation of acetate (as acetyl CoA) in the Krebs' cycle yielding a net of three NADH and one QH₂ for oxidative phosphorylation, as in *Escherichia coli* (Uندن 1999), two NADH and one QH₂ less the ATP (to activate the acetate) are available for growth with sulfoacetate. This is consistent with the markedly lowered energy supply reflected in the growth yield observed with sulfoacetate (4.4 g protein (mol C)⁻¹; Table 1) as compared with all other compounds (average 7.1 g protein (mol C)⁻¹).

The inducible pathways for the degradation of methanesulfonate or propanedisulfonate (Table 2) obviously do not involve sulfoacetaldehyde sulfo-lyase (Table 2, Fig. 3). Information on the degradation of methanesulfonate is available (Kelly and Murrell 1999; Reichenbecher and Murrell 2000), whereas the pathway for propanedisulfonate remains to be explored.

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