Sulphoacetaldehyde acetyltransferase yields acetyl phosphate: purification from *Alcaligenes defragrans* and gene clusters in taurine degradation

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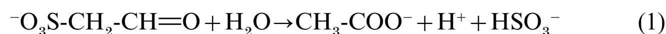
The facultatively anaerobic bacterium *Alcaligenes defragrans* NKNTAU was found to oxidize taurine (2-aminoethanesulphonate) with nitrate as the terminal electron acceptor. Taurine was transaminated to 2-sulphoacetaldehyde. This was not converted into sulphite and acetate by a 'sulphoacetaldehyde sulpho-lyase' (EC 4.4.1.12), but into sulphite and acetyl phosphate, which was identified by three methods. The enzyme, which required the addition of phosphate, thiamin diphosphate and Mg^{2+} ions for activity, was renamed sulphoacetaldehyde acetyltransferase (Xsc; EC 2.3.1.-). Inducible Xsc was expressed at high levels, and a three-step 11-fold purification yielded an essentially homogeneous soluble protein, which was a homotetramer in its native form; the molecular mass of the subunit was found to be between about 63 kDa (SDS/PAGE) and 65.3 kDa (matrix-assisted laser-desorption ionization–time-of-flight MS). The N-terminal and two internal amino acid sequences were determined, and PCR primers were generated. The *xsc* gene was amplified and sequenced; the derived molecular mass of the processed protein was 65.0 kDa. The downstream gene presumably encoded the inducible phosphate acetyltransferase (Pta) found in crude extracts. The desulphonative enzymes ('EC 4.4.1.12') from *Achromobacter xylosoxidans* NCIMB 10751 and *Desulfonispora thiosulfatigenes* GKNTAU were shown to be Xscs. We detected at least three subclasses of *xsc* in Proteobacteria and in Gram-positive bacteria, and they comprised a distinct group within the acetohydroxyacid synthase supergene family. Genome sequencing data revealed *xsc* genes in *Burkholderia fungorum* (80 % sequence identity) and *Sinorhizobium meliloti* (61 %) with closely linked *pta* genes. Different patterns of regulation for the transport and dissimilation of taurine were hypothesized for *S. meliloti* and *B. fungorum*.

Key words: desulphonation, phosphate acetyltransferase, sequence comparison, sulphoacetaldehyde sulpho-lyase, thiamin diphosphate.

INTRODUCTION

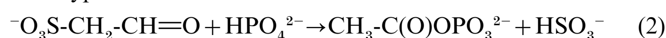
Sulphoacetaldehyde (Scheme 1) is the common intermediate in the dissimilation of the natural products taurine (2-aminoethanesulphonate), isethionate (2-hydroxyethanesulphonate) and 2-sulphoacetate, in both aerobic and anaerobic bacteria [1]; ethane-1,2-disulphonate is also degraded via sulphoacetaldehyde [2]. The compound is an intermediate in the biosynthesis of coenzyme M [3]. Sulphoacetaldehyde is, further, an intermediate in the assimilation of sulphonate sulphur from taurine in anaerobic bacteria [4,5], so for the first time, the same enzymic reaction has been found to be involved in desulphonation in both the assimilation of sulphonate sulphur and the dissimilation of sulphonate carbon (see [6]). The assimilation of sulphonate sulphur from taurine in aerobes (e.g. *Escherichia coli*) is catalysed by 2-oxoglutarate-dependent taurine dioxygenase ($TauD_{Ec}$; EC 1.14.11.17); transport of taurine is catalysed by an ABC transporter, $TauABC_{Ec}$ [7].

The cytoplasmic enzyme with the common name 'sulphoacetaldehyde sulpho-lyase' (EC 4.4.1.12) was believed to catalyse the desulphonation of sulphoacetaldehyde [2,5,8–14]. This enzyme has been purified only twice, from *Achromobacter xylosoxidans* with loosely bound thiamin diphosphate (ThDP) and a low affinity for sulphoacetaldehyde (K_m^{app}, 5 mM) [9], and from *Desulfonispora thiosulfatigenes* with tightly bound ThDP, a high affinity for sulphoacetaldehyde (0.2 mM) and an established subunit size (≈ 63 kDa) [15]. The reaction was considered to be:



$$^{-}O_3S\text{-}CH_2\text{-}CH{=}O + H_2O \rightarrow CH_3\text{-}COO^{-} + H^{+} + HSO_3^{-} \quad (1)$$

but we had reason to re-examine this conclusion, when a gene sequence, neighbouring that encoding the 'sulpho-lyase' in *Alcaligenes defragrans*, was found to encode a putative phosphate acetyltransferase (Pta; historically known as phosphotransacetylase; EC 2.3.1.8), which interconverts acetyl phosphate and acetyl-CoA. We had observed that the 'sulpho-lyase' from *D. thiosulfatigenes* was active in phosphate buffer only [15]. Further, the ThDP-dependent enzymes fructose-6-phosphate phosphoketolase (EC 4.1.2.22) and pyruvate oxidase (EC 1.2.3.3) generate acetyl phosphate. So we hypothesized that the desulphonative enzyme yielded acetyl phosphate, which is known to be labile [16], and which had presumably been hydrolysed in earlier work. The hypothesis was thus:



$$^{-}O_3S\text{-}CH_2\text{-}CH{=}O + HPO_4^{2-} \rightarrow CH_3\text{-}C(O)OPO_3^{2-} + HSO_3^{-} \quad (2)$$

This enzyme was tentatively called sulphoacetaldehyde acetyltransferase (Xsc; EC 2.3.1.-) and we proceeded to confirm the reaction in *Alc. defragrans* (Xsc_{Ad}), *Ach. xylosoxidans* (Xsc_{Ax})

Abbreviations used: DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; MALDI-TOF MS, matrix-assisted laser-desorption ionization–time-of-flight MS; ORF, open reading frame; *pta*, phosphate acetyltransferase gene; ThDP, thiamin diphosphate; *xsc*, sulphoacetaldehyde acetyltransferase gene.

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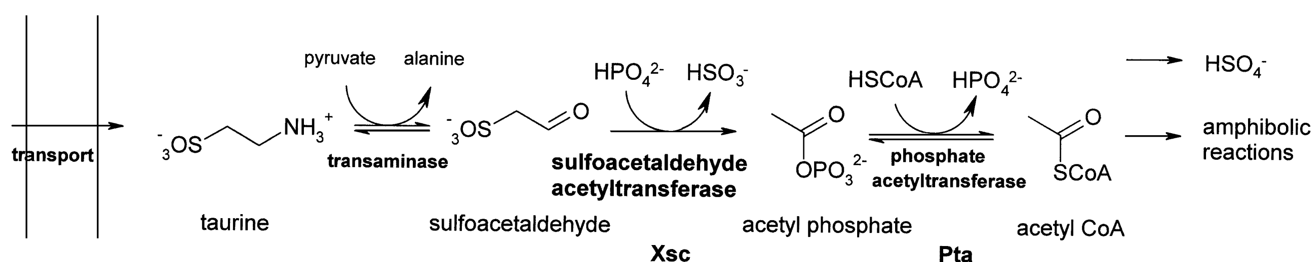
The nucleotide sequence data reported will appear in the GenBank Nucleotide Sequence Database under the following accession numbers: AY134843, *xsc* and *pta* from *Alcaligenes defragrans*; AY134844, *xsc* from *Achromobacter xylosoxidans*; AY134845, *xsc* and *pta* from *Burkholderia* sp. strain ICD; AY134846, *xsc* from *Ralstonia* sp. strain EDS1; AY134847, *xsc* from *Ralstonia* sp. strain EDS2; AY134848, *xsc* from *Comamonas* sp. strain SFCD1; AY134849, 16 S rDNA from *Burkholderia* sp. strain ICD; AY134850, 16 S rDNA from *Comamonas* sp. strain SFCD1; AY157621, 16 S rDNA from *Paracoccus denitrificans* NKNIS.



Scheme 1 The role of sulphoacetaldehyde acetyltransferase (Xsc) in the catabolism of taurine by *Alc. defragrans* NKNTAU

Transport systems are axiomatic for sulphonates, and some have been detected physiologically [1]. The first metabolic step in *Alc. defragrans* is transamination via taurine:pyruvate aminotransferase (EC 2.6.1.–) [17] to yield sulphoacetaldehyde and alanine; the enzyme is inducible (K. Denger, unpublished work). The organic product of the acetyltransferase, acetyl phosphate, is converted into acetyl-CoA by phosphate acetyltransferase (Pta) and presumably largely oxidized in the tricarboxylic acid cycle and the glyoxylate bypass; there is negligible acetate kinase in these cells (the present study). The inorganic product of the acetyltransferase reaction, sulphite, was not detected during growth of the organism [13]; we have detected a sulphite dehydrogenase [54], whose activity presumably explains the absence of sulphite in the growth medium and the presence of stoichiometric amounts of sulphate (K. Denger, unpublished work).

and *D. thiosulfatigenes* (Xsc_{Dt}). The abbreviation Xsc thus replaces the Sly (sulpho-lyase) used previously in [15].

The dissimilation of taurine has been reviewed [1] and found to require four steps to reach amphibolic pathways: firstly, a transport system to enter the cell (Scheme 1); secondly, conversion into sulphoacetaldehyde {usually via taurine:pyruvate aminotransferase (EC 2.6.1.-) [17] but a poorly-defined taurine dehydrogenase (EC 1.4.99.2) [18] was found in *Ach. xylosoxidans*}; thirdly, Xsc to generate acetyl phosphate; and fourthly, in organisms which utilize taurine as the source of electrons, Pta to generate acetyl-CoA for the Krebs' and glyoxylate cycles (Scheme 1). The fourth step is more complex in organisms whose metabolism involves the sulphonate moiety as an acceptor of electrons: Pta presumably converts acetyl phosphate from Xsc into acetyl-CoA for anabolism, whereas acetate kinase (EC 2.7.2.1) operates in substrate-level phosphorylation and acetate is excreted. The inducible degradative pathway (Scheme 1) may occur on its own, as in *Alc. defragrans* [13], or as one of several separately inducible pathways which converge at sulphoacetaldehyde, as in *Ralstonia* sp. strain EDS1 [2].

We now report that acetyl phosphate is the organic product generated quantitatively from sulphoacetaldehyde by the desulphonative enzyme from three bacteria. This establishes that Xsc is the correct assignment of EC 4.4.1.12, and emphasizes the importance of acetyl phosphate in metabolism. We describe the Xsc_{Ad} from *Alc. defragrans* grown with taurine in a nitrate respiration. The *xsc* gene is found in several subdivisions of the Proteobacteria, and in Gram-positive bacteria, and at least three subgroups can be recognized. Different patterns of dissimilative pathways from taurine via sulphoacetaldehyde to amphibolic pathways are known [1], and one complete pathway can now be postulated in the genome sequence of *Sinorhizobium meliloti*.

EXPERIMENTAL

Materials

Sulphoacetaldehyde dihydrate was synthesized as the bisulphite addition complex [15]. It was used directly in the experiments without conversion into the aldehyde (cf. [3]). CoA (> 95%; homogeneous by HPLC) and acetyl-CoA (> 99%; homogeneous by HPLC) were from Sigma (Deisenhofen, Germany); lithium potassium acetyl phosphate (97%) was from Fluka (Buchs, Switzerland). The commercial organosulphonates and general chemicals used were of high purity (about 99%) from Fisher Scientific (Ulm, Germany), Fluka, Merck (Munich, Germany) or Sigma-Aldrich. Gases (N_2, CO_2) were obtained from Messer Griesheim (Ludwigshafen, Germany).

Organisms and growth conditions

Alc. defragrans NKNTAU [DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) accession number 11046], *Bilophila wadsworthia* RZATAU (DSM 11045), *D. thiosulfatigenes* GKNTAU (DSM 11270), *Desulfovibrio* sp. strain RZACYSA, *Paracoccus denitrificans* NKNIS, *Paracoccus pantotrophus* NKNCYSA (DSM 12449), *Ralstonia* sp. strain EDS1 (DSM 13640), putative *Ralstonia* sp. strain EDS2 and *Rhodococcus opacus* ISO-5 (DSM 14600) were isolated in this laboratory [2,13,14,19–21]. *Desulfomicrobium norvegicum* (DSM 1741) [22] was obtained from the German Collection of Microorganisms (DSMZ), Braunschweig, Germany. *Ach. xylosoxidans* NCIMB 10751 [23] is available from NCIMB, Aberdeen, U.K. Two Gram-negative, environmental isolates, strains ICD and SFCD1 [11,12], were kindly made available by J. Quinn (Queen's University, Belfast, N. Ireland, U.K.). About 1500 bp of each 16 S rDNA gene was sequenced, its phylogenetic position evaluated with ARB software in the ARB database (http://www.arb-home.de/) and found to be compatible with simple physiological properties of the organisms. The organisms were then deposited as *Burkholderia* sp. strain ICD (DSM 15090) and *Comamonas* sp. strain SFCD1 (DSM 15091) with the DSMZ. The 16 S rDNA sequence of *Alcaligenes* sp. strain NKNTAU was re-evaluated with ARB software and attributed to *Alc. defragrans* (cf. [24]); the 16 S rDNA sequence of strain NKNIS [13] was attributed to *P. denitrificans*, compatible with physiological properties. *Burkholderia fungorum* LB400 and *Desulfitobacterium hafniense* DCB-2 were kindly supplied by J. Tiedje (University of Michigan, East Lansing, MI, U.S.A.). *S. meliloti* Rm1021 was kindly made available by S. Weidner and A. Pühler (University of Bielefeld, Bielefeld, Germany).

The growth media for organisms grown under anoxic conditions were based on a carbonate-buffered freshwater medium whose redox potential was set with titanium(III) nitrilotriacetate [14]. The denitrifiers *Alc. defragrans* NKNTAU and *P. pantotrophus* NKNCYSA were cultivated with nitrate (20 mM) as an electron acceptor and taurine (12 mM) as a source of carbon and electrons [13,20]. The medium for the taurine reducer *B. wadsworthia* RZATAU was supplemented with 1,4-naphthoquinone and contained formate (60 mM) as an electron donor and taurine

(20 mM) [19]. The sulphonate reducer *Desulfovibrio* sp. strain RZACYSA or *Des. norvegicum* was grown with lactate (20 mM) as an electron donor and taurine or isethionate, respectively (10 mM), as an electron acceptor. The sulphite reducer *De. hafniense* DCB-2 was grown with lactate (20 mM) as an electron donor, isethionate (10 mM) as the electron acceptor and yeast extract (0.1 %). The fermentative organism *D. thiosulfatigenes* GKNTAU used taurine (20 mM) as a sole source of carbon and energy [14]. Cultures were incubated under an atmosphere of N_2+CO_2 (80:20, v/v) at 30 °C in the dark.

The media for organisms grown under oxic conditions were based on a phosphate-buffered minimal medium [25] and contained, for *Ach. xylosoxidans*, 20 mM taurine plus 0.1 % (w/v) yeast extract [23], for *Burkholderia* sp. strain ICD, 20 mM isethionate [11], for *B. fungorum* LB400, 20 mM taurine, for *Comamonas* sp. strain SFCD1, 20 mM sulphoacetate or 20 mM isethionate [12], for *P. denitrificans* NKNIS, *Ralstonia* sp. strains EDS1 and EDS2, and *R. opacus* ISO-5, 20 mM taurine [2], and for *S. meliloti* Rm1021, 20 mM taurine. Cultures were incubated on a rotary shaker at 30 °C in the dark.

Preparation of cell-free extracts and enzyme assays

Cultures (e.g. 100 ml) were harvested oxically at the end of the exponential growth phase (15000 ***g***, 20 min, 4 °C), and the cells were washed twice with 100 mM potassium phosphate buffer, pH 7.5, containing 5 mM $MgCl_2$, resuspended in about 1 ml of 50 mM potassium phosphate buffer, pH 7.5, containing 5 mM $MgCl_2$, and disrupted by four passages through a chilled French pressure cell (140 MPa). Whole cells and debris were removed by centrifugation (15000 ***g***, 5 min, 25 °C), and the supernatant fluid was termed crude extract.

The yield of *Alc. defragrans* from a 10-litre culture was ≈ 9 g of cells (wet weight), which could be stored at −20 °C until required. Portions of cells (1.5 g) resuspended in 6 ml of buffer were used for enzyme separations. *D. thiosulfatigenes* was grown under conditions of taurine fermentation and the enzyme was purified as described previously [15]. *Ach. xylosoxidans* was grown aerobically with isethionate in the presence of yeast extract; the utilization of isethionate was not quantitative: the cells and extracts were worked up as with *Alc. defragrans* for purification of Xsc.

Xsc was assayed discontinuously. The reaction mixture (1 ml) at 30 °C routinely contained 100 μmol of potassium phosphate buffer, pH 7.5, 5 μmol of $MgCl_2$, 10 μmol of sulphoacetaldehyde bisulphate addition complex, 1 μmol of ThDP and 0.1–1.0 mg of protein, with which the reaction was started. The reaction was linear for several minutes. Negative controls were set up with boiled protein (10 min, 100 °C), without substrate [sulphoacetaldehyde or phosphate (50 mM Hepes buffer, pH 7.5, and desalted protein)] or without ThDP. Samples were taken at intervals. Work to establish the reaction catalysed by putative Xsc used up to six assay methods simultaneously: the disappearance of sulphoacetaldehyde, the formation of sulphite, the formation of acetic acid after hydrolysis of acetyl phosphate in acid, the formation of acetate, and chemical and enzymic determinations of acetyl phosphate. We initially allowed the degradation of sulphoacetaldehyde to reach completion and assayed the resultant putative acetyl phosphate enzymically. This assay was stoichiometric with Xsc_{Dt}, but the high K_m values and the low specific activities of Xsc_{Ax} and Xsc_{Ad} involved very long incubation times, during which acetyl phosphate was lost (cf. [16,26]). We thus chose to determine what portion of 4 mM sulphoacetaldehyde disappeared during short incubation times (3 min for Xsc_{Dt} and 10 min for Xsc_{Ax} and Xsc_{Ac}). We used the simplest routine enzyme assay in other sections of the work, formation of acetic acid by GC, but we considered this valid only after the formation of acetyl phosphate had been established. These assay conditions [11] were optimized for enzymes of Xsc subgroup 1 (see below), but they also detected the enzymes in subgroups 2 and 3. Optimized assay conditions for the enzyme in subgroup 2, in which subgroup 1 is inactive, were described elsewhere [15].

Pta was assayed photometrically by the formation of acetyl-CoA [27]. Acetate kinase was assayed discontinuously as the formation of acetyl phosphate [28]; desalted crude extract was used in the assay at a final concentration of 1–5 mg of protein/ml.

Purification of Xsc from *Alc. defragrans* and from *Ach. xylosoxidans*

Manipulations with crude extract were done on ice. Chromatographic separations were done at room temperature (≈ 20 °C) under air; the collected fractions were stored on ice.

Nucleic acids in crude extract were precipitated by addition of streptomycin sulphate (to 2 %) and removed together with membranous particles by ultracentrifugation (200000 ***g***, 30 min, 4 °C). The supernatant fluid was diluted 1:2.5 in distilled water, and the resulting precipitate was removed by centrifugation (15000 ***g***, 5 min, 25 °C). The supernatant fluid was loaded on to an anion-exchange column (Mono Q 10/10; Amersham Biosciences, Freiburg, Germany) equilibrated with 20 mM potassium phosphate buffer, pH 6.3, at 2 ml/min, and proteins were eluted with a gradient to 0.5 M Na_2SO_4 in buffer: buffer alone was pumped for 15 min, when a linear gradient to 0.075 M Na_2SO_4 over 10 min was started, thereafter the gradient was ramped to 0.225 M Na_2SO_4 over 35 min prior to salting proteins off the column and regenerating the initial conditions. Fractions of 2 ml were collected. Active fractions were pooled, diluted (1:5) in distilled water, and proteins were subjected to a second Mono Q chromatographic separation where the second step of the gradient was from 0.075 to 0.125 M Na_2SO_4 over 35 min. Xsc was separated from *Ach. xylosoxidans* by the same method.

Analytical methods

Sulphoacetaldehyde was derivatized and determined by HPLC [15,29]. Sulphite was quantified as the fuchsin complex [15]. Acetic acid was determined by GC [19]; the limit of detection was 0.05 mM, referring to the enzyme assay. Acetate was determined by the coupled reactions of acetyl-CoA synthase, citrate synthase and malate dehydrogenase, with the latter as preceding indicator reaction [30,31]; the enzymes were from Roche-Boehringer (Mannheim, Germany) and the limit of detection was 0.05 mM, referring to the Xsc assay. Acetyl phosphate was determined chemically as iron(III) acetyl hydroxamate [16,32]. Acetyl phosphate was determined enzymically by the reaction with CoA catalysed by Pta [33]. CoA and acetyl-CoA were separated and quantified by gradient reversed-phase HPLC (Nucleosil 5 C_{18}); a diode array detector was used. The mobile phase was 50 mM potassium phosphate buffer, pH 2.2 (0.5 ml/min), and a linear gradient to 60 % methanol in 6 min was initiated after 2 min; CoA and acetyl-CoA were eluted at 10.3 and 11.1 min respectively. Denatured proteins were separated by SDS/PAGE with gradient gels [34] and stained with colloidal Coomassie Brillant Blue G250 [35]. Protein was assayed by protein-dye binding [36]. Matrix-assisted laser-desorption ionization–time-of-flight MS (MALDI-TOF MS) with *trans*-ferulic acid as the matrix was done as described previously [37]. The N-terminal amino acid sequence

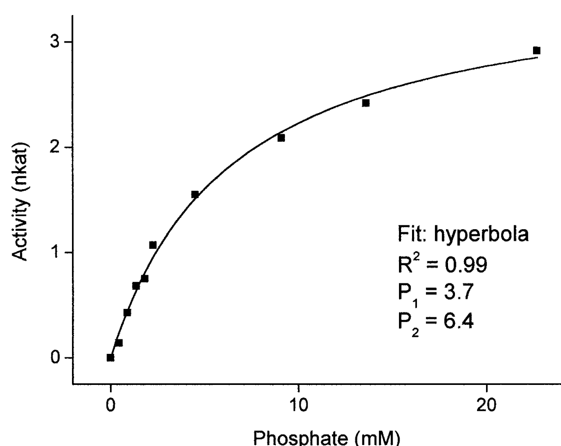


Figure 1 Reaction rate of the desulphonative enzyme, Xsc, from *Alc. defragrans* as a function of phosphate concentration

The reaction mixture contained 10 mM sulphoacetaldehyde ($5\times K_m^{app}$) and 1 mM ThDP ($25\times K_m^{app}$). The experimental data (■) are shown together with a hyperbola fitted by non-linear regression.

of a blotted protein was determined after Edman degradation, as indicated previously [38]. The sequences of internal peptides were obtained by MALDI-TOF MS after proteolysis (lysyl endopeptidase; EC 3.4.21.50) of purified protein and separation of the peptides by HPLC (Top-Lab Service Facility, Munich, Germany). Dynamic light-scattering was described elsewhere [15].

Analytical gel-filtration chromatography (Superose 12 HR 10/30; Amersham Biosciences) of the purified Xsc was done after concentration and rebuffering (Centricon 10; Amicon, Millipore, Bedford, MA, U.S.A.). The column was equilibrated at a flow rate of 0.4 ml/min with 20 mM potassium phosphate buffer, pH 6.3, containing 150 mM Na_2SO_4.

Xsc has multiple substrates (see eqn 2, above), which complicates the representation of enzyme kinetic data (e.g. [39]). We generated simple plots of rate versus substrate concentration and fitted hyperbolic curves to these raw data (cf. Figure 1, see below) in Microcal Origin 6.0 software to derive values of apparent K_m (K_m^{app}) for each substrate.

PCR, cycle sequencing and use of the Universal GenomeWalker™ Kit (Clontech) were described elsewhere [15]. PCR amplification of gene-specific DNA fragments was done with the following primers: degenerate primers derived from the N-terminal and internal amino acid sequences of Xsc_{Ad} (xsc-ad-Nu, GGIGGIGTICARGARATGAC; xsc-ad-intl, TTRCAYTCYTCIACIGCRTCICCCAT); degenerate primer derived from N-terminal sequence of Xsc_{Ax} (xsc-ax-Nu, ATGGCIGCIACITGYAAYIG); specific primers from conserved 5′ and 3′ regions of *xsc* genes in subgroup I (xsc-sgI-5u, GGCCCGCAGGCCATGACSCC; xsc-sgI-3l, TCAGACGTAGTCCTTGTACTTGTCGAGCA). Sequence analysis was done using the DNASTAR Lasergene programme package. The National Center for Biotechnology Information BLAST programs were used to search for similarities amongst the new and established sequences [40]. The PROSITE tools of the Swiss Institute of Bioinformatics were used to search for motifs. The sequence data for *B. fungorum* and *De. hafniense* have been provided freely by the U.S. Department of Energy Joint Genome Institute (http://www.jgi.doe.gov/JGI_microbial/html/index.html) for use in this publication only.

RESULTS

Formation of acetyl phosphate from sulphoacetaldehyde in three representative organisms

The desulphonative enzyme in desalted crude extracts from *Alc. defragrans* generated sulphite from 2-sulphoacetaldehyde (Table 1), as anticipated [9,15]. If the organic product was assayed after acidification, acetic acid was detected (Table 1), as anticipated [9,15], but when acetate was determined enzymically with no acidification step, negligible amounts were found (Table 1). The latter samples contained a compound which reacted with hydroxylamine to give putative acetyl hydroxamate, which was visualized as the Fe(III) chelate. Acetate did not react with hydroxylamine, as reported previously [26]. The yield of acetyl hydroxamate indicated quantitative recovery of the carbon moiety of the sulphoacetaldehyde as acetyl phosphate (Table 1). The desulphonative enzymes from *Ach. xylosoxidans* and *D. thiosulfatigenes* showed identical behaviour (Table 1). Less extensive experiments with the three purified enzymes confirmed that each one catalysed the reaction. Boiled enzyme was inactive.

Putative acetyl phosphate from the reaction of the desulphonative enzyme with sulphoacetaldehyde reacted with commercial Pta in the presence of CoA to yield acetyl-CoA. The latter was determined photometrically, and identified by co-chromatography with authentic material on reversed-phase HPLC and by its UV spectrum (λ_{min}, 229 nm; λ_{max}, 257 nm). The recovery of acetyl-CoA in the enzyme reaction represented equimolar recovery of the carbon moiety consumed in the desulphonation of sulphoacetaldehyde.

We confirmed that the organic product of the desulphonative enzyme was acetyl phosphate by MALDI-TOF MS. Analyses in the negative-ion mode gave signals at m/z 139 and 79: we interpreted them to be $[M-H]^-$ and PO_3^- respectively; the molecular mass of acetyl phosphoric acid is 140 Da. Authentic acetyl phosphate gave the same signals; the matrix and the negative controls, enzyme without organic substrate and substrate without enzyme, gave no such signals.

There was an absolute requirement for phosphate in the desulphonation reaction (Table 1): the enzyme from three sources gave no reaction in its absence. The reaction rate was a hyperbolic function of the phosphate concentration (Figure 1), and curve fitting allowed a value for K_{mPi}^{app} of 6.4 mM, to be calculated. Similar values (5–6 mM; Table 1) for the K_{mPi}^{app} of the enzyme were observed with the other enzymes. One of the enzymes was from *Ach. xylosoxidans* NCIMB 10751, the organism from which the desulphonative enzyme was originally purified [9]. We conclude that each of these enzymes catalyses the same ThDP-dependent reaction in which 1 mol each of sulphoacetaldehyde and phosphate is converted into 1 mol each of sulphite and acetyl phosphate (Table 1 and Scheme 1), as suggested in eqn (2).

We infer that the reaction catalysed by the ThDP-dependent desulphonative enzyme resembles the standard formation of 'active aldehyde' (e.g. [39]) and a detailed hypothesis is shown elsewhere [1]. The overall reaction, which determines enzyme nomenclature [41], is the transfer (and isomerization) of the oxoethyl group from the substrate to phosphate, yielding acetyl phosphate. This is likely to put it into the IUBMB's EC group 2, the transferases. The systematic name of the enzyme might be acetyl-phosphate:sulphite acetyltransferase (isomerizing) and the common name Xsc.

Pta was inducible in *Alc. defragrans*, being found at 4 mkat/kg of protein in extracts of taurine-grown cells and 0.5 mkat/kg of protein in extracts of acetate-grown cells; no acetate kinase was detected. These data support the pathway shown in Scheme 1. We found Pta and acetate kinase to be present in *D. thio-*

Table 1 The requirement for phosphate, values of K_m^{app} and the products formed from the transformation of $\approx$ 1 mM sulphoacetaldehyde by the desulphonative enzyme Xsc from *Alc. defragrans*, *D. thiosulfatigenes* and *Ach. xylosoxidans*

The enzyme was supplied as desalted crude extract. The data for stoichiometry are from one typical experiment. Each value is the mean of three determinations. Sulphoacetaldehyde was supplied as the bisulphite addition complex; net sulphite refers to cleavage of the non-complexed molecule, i.e. half of the total. The values for S.D. varied from $< 6\%$ (sulphite, acetyl phosphate and acetic acid) and $< 20\%$ (sulphoacetaldehyde, measured by difference between initial and final concentrations) to $< 30\%$ (acetate). 'Acetate' was determined in samples that were not acidified, whereas 'acetic acid' was determined in the same samples after acidification. SAA, sulphoacetaldehyde.

Source of enzyme	Requirement for phosphate	K_m^{app} (mM)			Net sulphite (mM)	Acetate (mM)	Acetyl phosphate (mM)	SAA utilized (mM)	Acetic acid (mM)
		P_i	SAA	ThDP					
Alc. defragrans	Present	6.4*	2.1†	0.0022‡	1.1	0.2	1.3	1.3	1.0
D. thiosulfatigenes	Present	5.7§	0.2¶	None‖	0.9	0.0	1.2	1.1	0.9
Ach. xylosoxidans	Present	4.8*	5.0**	0.0027**	1.2	0.1	1.4	1.0	1.0

* Sulphoacetaldehyde was present at $\approx 1.5\text{–}4 \times K_m^{app}$, ThDP at $\approx 40 \times K_m^{app}$.
† Phosphate was present at $\approx 8 \times K_m^{app}$, ThDP at $\approx 40 \times K_m^{app}$.
‡ Phosphate was present at $\approx 8 \times K_m^{app}$, sulphoacetaldehyde at $\approx 4 \times K_m^{app}$.
§ Sulphoacetaldehyde was present at $\approx 50 \times K_m^{app}$.
¶ Data recalculated from Denger et al. [15].
‖ ThDP tightly bound to enzyme [15].
** Values from Kondo et al. [9], who believed them to be true K_m data. The experiments were done with > 7 values and the regression value (R^2) was > 0.97.

sulfatigenes at levels of 95 and 40 mkat/kg of protein, respectively, as discussed for anaerobic organisms which catalyse reduction of, for example, taurine [1]. We detected no Pta or acetate kinase in *Ach. xylosoxidans*, but all other organisms tested contained Pta (see below), and we wonder whether *Ach. xylosoxidans* contains another class of Pta.

Xsc in crude extracts of bacteria

Crude extracts of *Alc. defragrans* NKNTAU grown with taurine under denitrifying conditions contained Xsc activity (3–5 mkat/kg of protein): negligible activity was detected in extracts of cells grown with acetate. The presence of the acetyltransferase always coincided with the expression of a 63 kDa protein, which was found in the soluble fraction of the cell (Figure 2, lane 3). This inducible protein was shown to be Xsc (see below).

We used the same assay conditions for extracts of 12 other taurine- (or isethionate- or sulphoacetate-) utilizing Gram-negative bacteria in the α-, β- and δ-Proteobacteria (Table 2). Extracts from 10 of these organisms showed inducible formation of Xsc (1–5 mkat/kg of protein) and, where tested, an inducible 63 kDa protein. Two δ-Proteobacteria, *B. wadsworthia* and *Des. norvegicum*, showed neither activity nor an inducible 63 kDa protein. *D. thiosulfatigenes* is a Gram-positive organism that expresses Xsc_{Dt}. Where tested (6 of 10 extracts from Gram-negative bacteria), no activity was detected with the assay optimized for Xsc_{Dt}. We thus confirm [15] that there are at least two biochemical subgroups of acetyltransferases; the group discovered by Kondo and Ishimoto [8], which is obviously widespread, and which we term subgroup 1 (Table 2), and that found in strain *D. thiosulfatigene*s GKNTAU and termed subgroup 2 (Table 2). One other taurine-dissimilating Gram-positive organism was examined, *R. opacus*, and it expressed inducible Xsc (Table 2).

Purification and properties of Xsc from *Alc. defragrans* NKNTAU

The initial two steps in the enzyme purification, precipitation/centrifugation and dilution, allowed 55 % of the total protein to be removed (Table 3). Xsc_{Ad} was eluted from the first anion-exchange step as a sharp peak at 0.12 M Na_2SO_4. The second

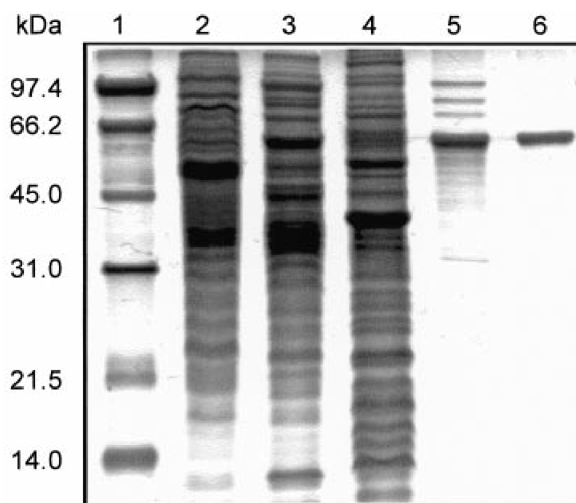


Figure 2 SDS/PAGE of *Alc. defragrans* NKNTAU fractions

Different protein fractions of *Alc. defragrans* NKNTAU grown under inducing or non-inducing conditions or from the enzyme purification were separated by SDS/PAGE (12% gel) and stained with colloidal Coomassie Brilliant Blue G250. Lane 1, molecular-mass standards (97.4 kDa, phosphorylase *b*; 66.2 kDa, BSA; 45.0 kDa, ovalbumin; 31.0 kDa, carbonic anhydrase; 21.5 kDa, trypsin inhibitor; 14.4 kDa, lysozyme); lane 2, soluble fraction after growth with acetate (non-induced; 30 μg); lane 3, soluble fraction after growth with taurine (30 μg); lane 4, membrane fraction after growth with taurine (30 μg); lane 5, fraction after first anion-exchange chromatography (4 μg); lane 6, fraction after second anion-exchange chromatography (4 μg).

separation on the anion-exchange column yielded a preparation with negligible impurities (Figure 2). The purification factor we obtained was 11, with a yield of 23 %, where the most significant losses were in the anion-exchange steps. This was in part due to poor enzyme stability at pH 6.3, which facilitated the separation; the enzyme was more stable in extraction buffer.

The activity of the enzyme was largely independent of buffer and pH value from 6.0 to 8.2, above which the organic substrate began to decay spontaneously. There was somewhat lower activity (about 75 %) in 50 mM citrate or 50 mM Mes buffer

Table 2 Detection of Xsc as activity or an inducible protein in bacteria, which dissimilate taurine, isethionate or sulphoacetate, or as *xsc* in databases

Organism	Reference	Phylogeny	Xsc subgroup in extract	63 kDa protein?	G + C content of *xsc* (%)
P. denitrificans NKNIS	This study	Rhodobacteraceae (α)	Present*	+	No data
P. pantotrophus NKNCYSA	[20]	Rhodobacteraceae (α)	Present*	+	No data
Rhodobacter capsulatus SB1003	[5]	Rhodobacteraceae (α)	Present†	No data	68 (*in silico*)
S. meliloti Rm1021	[53]	Rhizobiaceae (α)	Present*	No data	64 (*in silico*)
Ach. xylosoxidans NCIMB 10751	[9]	Alcaligenaceae (β)	1	+	64
Alc. defragrans NKNTAU	This study	Alcaligenaceae (β)	1	+	66
B. fungorum LB400	JGI‡	Burkholderia/Ralstonia (β)	Present*	No data	65 (*in silico*)
Burkholderia sp. strain ICD	[11]	Burkholderia/Ralstonia (β)	1	+	65
Comamonas sp. strain SFCD1	[12]	Comamonadaceae (β)	1	+	68
Ralstonia sp. strain EDS1	[2]	Burkholderia/Ralstonia (β)	1	+	64
Ralstonia sp. strain EDS2	[2]	Burkholderia/Ralstonia (β)	Present*	No data	63
Clone P1023 (*Leishmania major*?)		Bacterium (β?)	No data	No data	67 (*in silico*)
Desulfovibrio sp. strain RZACYSA	[19]	Sulphate reducers (δ)	Present*	+	No data
Des. norvegicum DSM 1741	[22]	Sulphate reducers (δ)	None	None	N/A
B. wadsworthia RZATAU	[19]	Sulphate reducers (δ)	None	None	N/A
De. hafniense DCB-2	JGI‡	Peptococcaceae	No data	No data	49 (*in silico*)
D. thiosulfatigenes GKNTAU	[14]	Peptococcaceae	2	+	39§
R. opacus ISO-5	[21]	Nocardiaceae	Present*	+	No data

* Enzyme activity was detected in cell extracts, but not characterized further.
† The gene product was active in whole cells [5].
‡ U.S. Department of Energy Joint Genomic Institute preliminary sequence data (see text).
§ Data from [15].

Table 3 Purification of Xsc from *Alc. defragrans* NKNTAU

Step	Protein (mg)	Activity (nkat)	Specific activity (mkat/kg)	Yield (%)	Purification factor
Crude extract	83.6	287	3.4	100	1.0
Soluble fraction	48.8	242	5.0	84	1.4
Supernatant after dilution	37.4	233	6.2	81	1.8
First anion exchange	11.5	131	11.4	46	3.3
Second anion exchange	1.7	65	38.2	23	11.2

containing 50 mM phosphate at pH 6.0 and 50 mM Mops buffer containing 50 mM phosphate at pH 6.5, than in 50 mM Tris or 50 mM Hepes containing 50 mM phosphate at pH 7.5 (about 90 %), and 50 mM phosphate at pH 7.5 and 50 mM Tris containing 50 mM phosphate at pH 8.2 (about 100 %). There was a broad apparent temperature optimum at about 30 °C.

The reaction was stoichiometric (Table 1). The K_m^{app} values for sulphoacetaldehyde, phosphate and ThDP were about 2.1 mM ($n = 6$, $R^2 > 0.97$), 6.4 mM ($n = 10$, $R^2 > 0.99$) and 2.2 μM ($n = 11$, $R^2 > 0.99$), respectively. The corresponding V_{max} values were 3, 4 and 17 mkat/kg of protein, respectively.

The purified enzyme was chromatographed on a calibrated gel-filtration column, and a molecular mass of 252 kDa (± 5 %) for the native protein was determined. This value was confirmed by dynamic light scattering (247 kDa). The molecular mass of the denatured protein was about 63 kDa (Figure 2), with a unique N-terminal amino acid sequence (see below), and this correlated reasonably with a value of 65.0–65.3 kDa by MALDI-TOF MS. We conclude that the native protein is a homotetramer.

The sequence of the N-terminal amino acids was ANDT-RQVVQGVQEMTPSEAF. There was no evidence for ambiguity in this sequence, so there was only one type of subunit. This sequence shared no logical similarity with entries in the databases. The enzyme was subjected to proteolysis, and two internal fragments, FPVIISGGGVVMGDAVEECK and NARIIQVD-ADSK, were sequenced: these sequences shared significant similarity with acetohydroxyacid synthases. The protein micro-sequences allowed us to deduce primer pairs to amplify segments of the *xsc* gene.

The enzyme from *Ach. xylosoxidans*, Xsc_{Ax}, was an inducible protein whose molecular mass under denaturing conditions was about 64 kDa, as indicated in Table 2. An analysis of the N-terminal amino acids yielded a unique sequence, AATDNR-KVVGGVAKMTPSEA, which indicated that there was only one type of subunit. The native enzyme eluted from the gel-filtration column as a double peak which we interpolated to represent 155 and 256 kDa, so we presumed that the native enzyme could be present as both a dimer and a tetramer.

Gene sequences

The PCR primer pairs allowed us to amplify and sequence central portions of the xsc_{Ad} gene; application of the Genome-Walker™ kit enabled us to sequence past the 5′- and 3′-ends of the gene. We detected stop codons in the 400 bp upstream of *xsc*, but no sequence attributable to a gene. Downstream (22 bp) of the *xsc* gene, we found an open reading frame (ORF; 969 bp, accession number AX134843), whose derived amino acid sequence shared 53 % identity with Pta from *P. denitrificans* (accession number P39197). This *pta* gene overlapped (32 bp) the 3′-end of an ORF on the complementary strand. The polypeptide derived from 828 bp of the complementary strand shared 42 % identity with a CobT (accession number AAA25793.1) [42].

The amino acid sequence derived from the xsc_{Ad} gene included the N-terminal sequence determined by Edman degradation (Figure 3A) and showed that the N-terminal methionyl group had been cleaved; the internal fragments (see above) were also present (results not shown). The derived molecular mass of the processed polypeptide was 65.0 kDa, within the range observed for the purified protein by MS (see above). This confirmed that we had sequenced the correct gene.

We compared by BLAST searches both the nucleotide sequence of xsc_{Ad} and the deduced amino acid sequence with annotated databases and incompletely sequenced genomes.

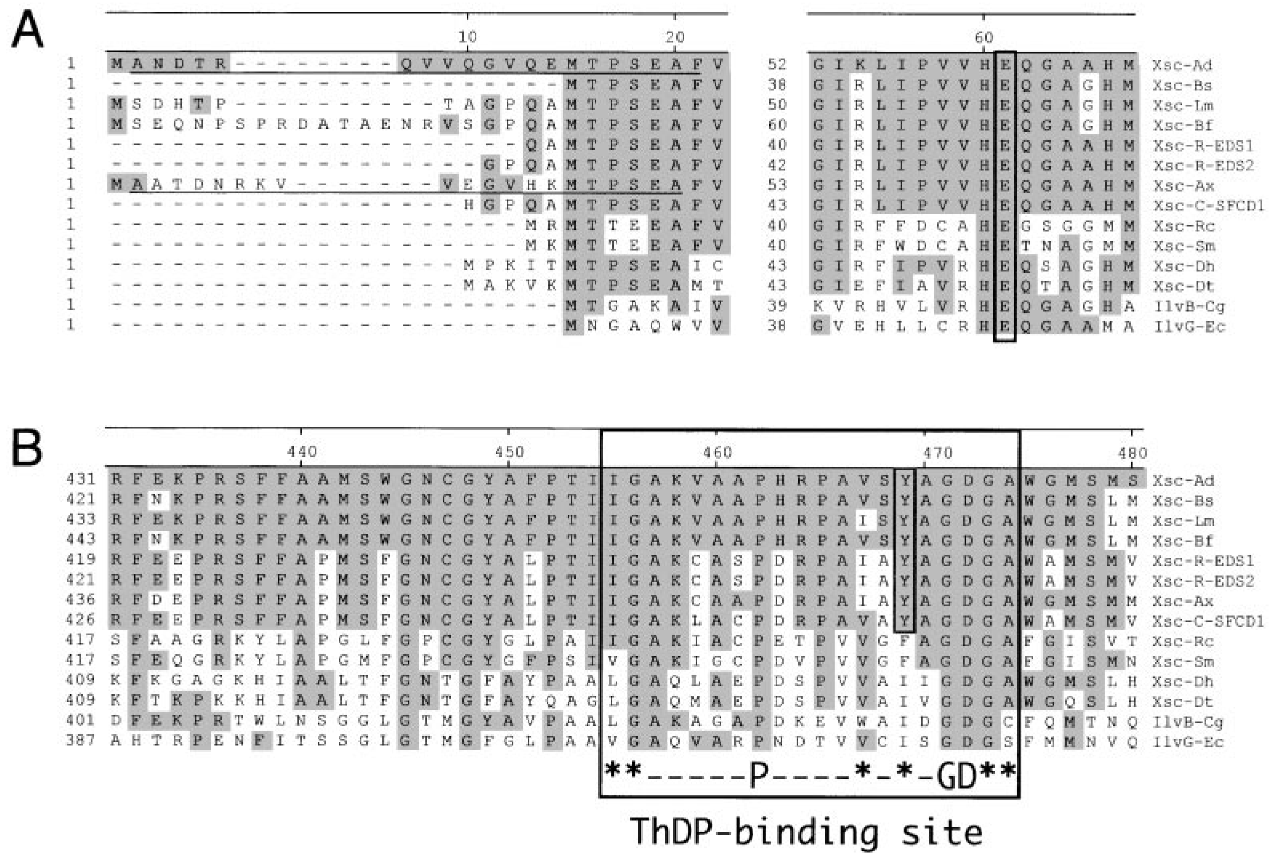


Figure 3 The derived sequences of amino acids at the N-termini (A) and the ThDP-pyrophosphate-binding sites (B) of 12 Xscs and two acetohydroxyacid synthases (Ilv)

The N-terminal amino acids (Edman degradation) determined for two enzymes in this paper are shown underlined; we detected two errors in the protein microsequence of Xsc_{Ax}, but the derived amino acid is shown here. The letters appended after the abbreviation of the gene-product give the organism involved: Ad, *Alc. defragrans*; Ax, *Ach. xylosoxidans*; Bs, *Burkholderia* sp. strain ICD; Bf, *B. fungorum*; Cg, *Corynebacterium glutamicum*; C-SFCD1, *Comamonas* sp. strain SFCD1; Dh, *De. hafniense*; Dt, *D. thiosulfatigenes*; Ec, *E. coli*; Lm, contaminative bacterial DNA in the *Leishmania major* sequencing programme; Rc, *Rhodobacter capsulatus*; R-EDS1, *Ralstonia* sp. strain EDS1; R-EDS2, *Ralstonia* sp. strain EDS2; Sm, *S. meliloti*.

Homologues of the *xsc* gene were found in the genome of *B. fungorum* LB400 (80 % identity with *ilvB*2; contig 432 gene 96), in bacterial contaminative DNA in the sequencing project for *Leishmania major* (82 % identity, no annotation, position 71 070–72 872; accession number AC091510.5), in the pSymB megaplasmid of *S. meliloti* Rm1021 (61 % identity with *ilvB*2; gene SMb21530) and in *Rhodobacter capsulatus* (61 % identity with orf590; cf. [5]). Lower levels of identity were found with the confirmed *ilvG* gene (acetohydroxyacid synthase) of *E. coli* (accession number AAC73188.1) and with the *ilvB* (acetohydroxyacid synthase) gene of *Corynebacterium glutamicum* (accession number AAC73188.1). The nearest non-redundant homologue of Xsc_{Ad} was Xsc_{Dt} (see the Introduction), which was not detected at the nucleotide level. A homologue of xsc_{Dt} was found in work in progress on the *De. hafniense* DCB-2 genome (Table 2).

B. fungorum LB400 and *S. meliloti* Rm1021 were both found to utilize taurine as the sole source of carbon and energy for aerobic growth, and Xsc was present (Table 2). This was interpreted as strong evidence that the annotation IlvB was incorrect, and we use the hybrid term e.g. IlvB-Xsc_{Bf} (for the enzyme in *B. fungorum*) to presage a possible alteration in the annotation. The derived sequence closest to IlvB-Xsc_{Sm} (from *S. meliloti* Rm1021) is Xsc from *R. capsulatus* (see above), whose function as Xsc was confirmed elsewhere [5].

The sequences of xsc_{Ad}, *ilvB*-xsc_{Bf} and xsc_{Lm} (from *L. major*) were aligned, which allowed us to derive PCR primer pairs from conserved regions. Amplification with these primer pairs gave specific products from the β-Proteobacteria only (Table 2); primer pairs from xsc_{Dt} gave no PCR products with any other tested organism (Table 2). The specific products were sequenced, and the sequences were extended by primer-walking PCR and cycle sequencing. It was possible to determine the (conserved) 3′-sequence of each of these genes, but some terminal 5′-sequences are missing, presumably due to micro-divergency (Figure 3; these four genes are marked in Figure 5, see below). We could confirm that the putative xsc_{Ax} gene sequence encoded Xsc_{Ax}, because the derived N-terminal sequence matched that from Edman degradation of the purified protein (Figure 3). The high levels of identity near the N-terminus of the Xsc proteins (Figure 3) are continued almost throughout, including the pyrophosphate-binding domain for ThDP (Figure 3); there are two major sources of divergence, the N-terminus itself (Figure 3) and a region near amino acid 400 (results not shown). All (derived) Xsc sequences, with those for two characterized acetohydroxyacid synthases (IlvG, IlvB), were used to generate a

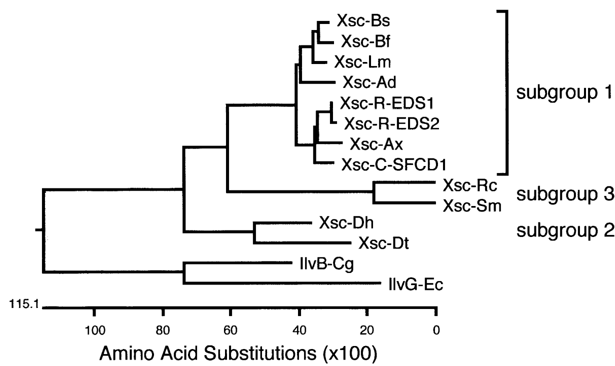


Figure 4 Xscs form three subgroups independent of the acetohydroxyacid synthases

The deduced amino acid sequences of the gene products were processed in ClustalW software to generate a phylogenetic tree. The letters appended after the abbreviation of the gene give the organism involved, as in Figure 3.

dendrogram. The Xsc enzymes were found in three subgroups (1–3), which were clearly separate from the acetohydroxyacid synthases (Figure 4).

We observed a putative pta_{Ad} immediately downstream of inducible xsc_{Ad} (see above), and we presume that this pta_{Ad} encodes the inducible Pta measured during growth with taurine (see above). The genomic locations of *ilvB*-xsc_{Bf}, *ilvB*-xsc_{Sm} and xsc_{Lm} all include a *pta* gene (Figure 5). *B. fungorum* and *S. meliloti* grown with taurine both expressed Pta (2.8 and 1.6 mkat/kg of protein, respectively). We found a putative *pta* gene downstream of xsc_{Bs} in *Burkholderia* sp. strain ICD and activity was detected (2.2 mkat/kg of protein). We then used PCR primers, which amplified a 1.2 kb fragment that overlapped xsc_{Ad} and pta_{Ad}; a fragment of the same length was obtained from *Ralstonia* sp. strains EDS1 and EDS2. All these *pta* genes share 54–60 % identity of sequence, independent of the subgroup of the neighbouring *xsc* gene. We presume that these organisms also encode Pta adjacent to Xsc. No signal for *pta* was obtained from *Ach. xylosoxidans*, which expresses no measurable Pta (see above).

DISCUSSION

Acetyl phosphate as the reaction product and its significance

The evidence presented here, which proves that acetyl phosphate is the organic product of the desulphonation reaction, is based on six complementary sets of data. Firstly, the product is reactive enough to form acetyl hydroxamate in the presence of hydroxylamine (cf. [26]), and only acetyl phosphate is feasible under the conditions that we used. Secondly, the putative acetyl phosphate reacts with Pta in the presence of CoA, a reaction which is known to be specific [33]. Thirdly, the acetyl-CoA formed in this enzymic reaction could be identified chromatographically and by its UV spectrum. Fourthly, the unknown was confirmed to be acetyl phosphate by MALDI-TOF MS. The fifth line of evidence involves the absolute requirement for phosphate in the reaction, together with the hyperbolic reaction kinetics (Figure 1), which indicate that this phosphate takes part in the reaction. Sixthly, the previously supposed product, acetate, is found in negligible amounts in samples which have not been mis-treated (Table 1).

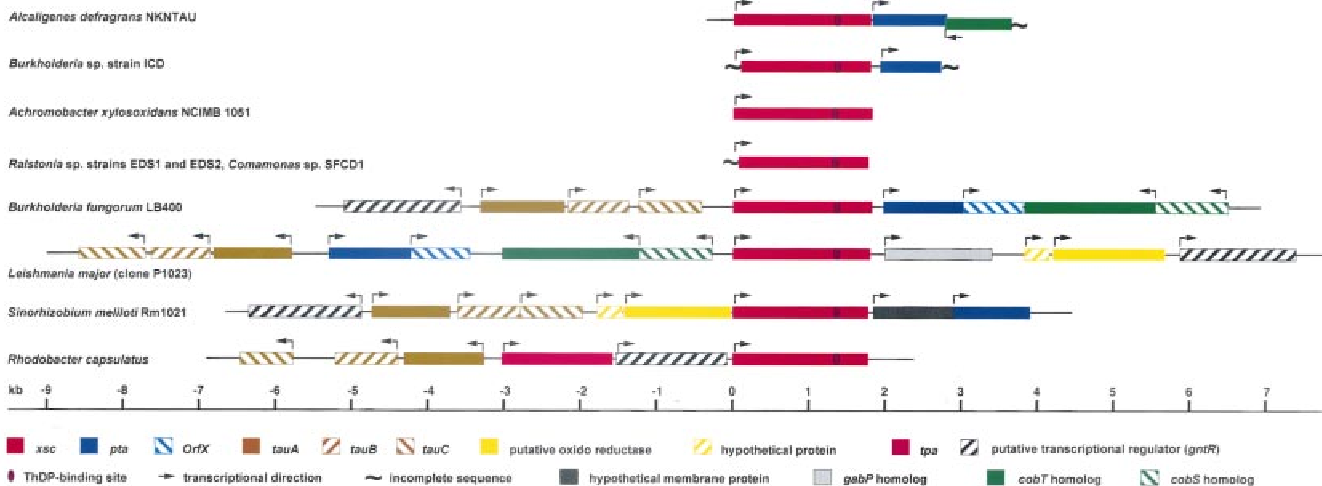


Figure 5 The genomic loci of some *xsc* genes

The common factor in these sequences is the *xsc* gene with the ThDP-pyrophosphate-binding site. Most loci have a *pta* gene in the immediate neighbourhood of *xsc*, analogous to the finding in *Alc. defragrans*; *Ach. xylosoxidans* is one exception (see the text), and the data from *R. capsulatus* concern sulphur assimilation, and may not require Pta. The third ORF found in the β-proteobacterium *Alc. defragrans* is on the complementary strand, and it is seen on the complementary strand of the β-proteobacterium *B. fungorum* and of the contaminative, putative β-proteobacterial DNA from the *L. major* sequencing project. These homologues of *cobT* are no more closely related to one another than to *cobT*; *cobST* seem to be encoded separately from the main *cob* cluster(s) [42]. Genes with homologies to $tauABC_{\mathrm{Ec}}$, but poorly grouped in a dendrogram, are found near each *xsc* gene found in genome sequencing projects. The putative taurine dehydrogenase encoded in *S. meliloti* (see the text; possibly with helper protein) has a close homologue on the '*L. major*' clone. The gene hypothesized to encode a regulator of taurine degradation in *S. meliloti* has as its nearest homologue the proposed regulator in *B. fungorum*; strangely, another putative regulator of this class is found in the sulphur assimilation genes of *R. capsulatus*, and yet another on the contaminant DNA fragment in '*L. major*'. The *xsc* gene of *De. hafniense* is mentioned in the text, but as the gene is located at the end of a short contig (July 2002), no further data are available.

This finding is in apparent disagreement with data collected over 30 years on at least three continents [2,8,10–12,15,43]. However, acetyl phosphate is known to be "very unstable at low and high pH and at elevated temperatures" [16]. Kondo and Ishimoto [8] studied *Ach. xylosoxidans* NCIMB 10751, worked exclusively with phosphate buffer in the enzyme assay and routinely measured sulphite as a reaction product. Acetate was measured as acetic acid by GC after acidification, as acetate by paper chromatography under alkaline conditions, and as acetyl phosphate photometrically after reaction with acetate kinase: in the first two methods, any acetyl phosphate would be quantitatively hydrolysed to the acetate that was detected, whereas the enzymic method used the zero-time sample as the control and would not distinguish between free acetate and acetyl phosphate. Shimamoto and Berk [10] studied *Pseudomonas aeruginosa* TAU5. They used exclusively phosphate buffer in the enzyme assay. Enzyme reactions were stopped by boiling the reaction mixture, which presumably hydrolysed any acetyl phosphate. Samples were then either acidified, to determine acetate as acetic acid by GC, or treated with alkali for paper chromatography as acetate. The extensive identifications of acetate were done after preparative paper chromatography. King et al. [11,12] worked with *Burkholderia* sp., *Aureobacterium* sp. and *Comamonas* sp., and Denger et al. [2,15] worked with *D. thiosulfatigenes* and *Ralstonia* sp. All enzyme assays involved phosphate buffer and acidification prior to finding acetic acid, so any acetyl phosphate would have been missed. Acetate was considered to be a product from taurine in *R. opacus* TCNS94 [43], but the HPLC determination involved an acidic mobile phase, so any acetyl phosphate would be hydrolysed to the acetate which was detected. *R. opacus* ISO-5 expresses an Xsc which yields acetyl phosphate (Table 2).

We prove in this paper that the desulphonative enzyme from *Ach. xylosoxidans* NCIMB 10751 and the enzyme from *D. thiosulfatigenes* catalyse formation of acetyl phosphate. Negligible acetate was detected. So the published work [8,15] is inaccurate in this detail. The enzyme purified by Kondo and Ishimoto [9] was essentially homogeneous; no protein standards were used, and we can now report that the subunit has a molecular mass of about 64 kDa. The native enzyme seems to be present in two species, the homodimer and the homotetramer. We suspect that some problem occurred during gel-filtration chromatography (85 kDa) in the original research [9]; the ThDP fold in ThDP-dependent enzymes is positioned between two subunits and requires at least a dimeric structure [44].

The biological significance of Xsc is at least 3-fold. Firstly, the reaction is a desulphonation (Scheme 1), which seems not to be present in mammals [45]; this is unchanged since its discovery by Kondo and Ishimoto [8]. Secondly, a high-energy phosphate bond is generated in the degradative pathway. We are used to this in glycolysis, where phosphoenolpyruvate is known as a "key metabolic intermediate" [39]. Acetyl phosphate, in contrast, is referred to as an "energy storage compound" [39]. The latter description possibly underestimates the significance of acetyl phosphate. We presume [1] that aerobes and nitrate reducers which degrade the widespread precursors of sulphoacetaldehyde, namely taurine and derivatives, isethionate and sulphoacetate from the plant sulpholipid, are largely dissimilated via acetyl phosphate, Pta (Scheme 1) and the tricarboxylic acid and glyoxylate cycles. We presume that fermentation of these sulphonates involves substrate-level phosphorylation via acetate kinase with excretion of acetate, together with anabolism via Pta [1]. Fermentation of sugars to yield acetyl phosphate via phosphoketolase, as in *Bifidobacterium* sp. [46], presumably also uses acetate kinase during formation of acetate and Pta for anabolism. *Lactobacillus* spp. dissimilating pyruvate via pyruvate oxidase [47] could well do the same. Indeed, acetyl phosphate is routinely involved in the excretion of acetate in bacterial fermentations and often in the activation of acetate prior to anabolism or catabolism in Archaea and some bacteria [48]. A third role of acetyl phosphate is its involvement in phosphorylation of two-component regulators in an increasing number of organisms (e.g. [49,50]).

Xsc and deduction of degradative pathways

Xsc_{Ad} is present in taurine-grown cells at 3.4 mkat/kg of protein (Table 3). This specific activity is sufficient to account for the growth rate of $0.07\ h^{-1}$ with a molar growth yield of 14 g of protein/mol of taurine [13], i.e. 1.4 mkat/kg of protein. The organism must invest some 9% of total protein (purification factor 11; Table 3) in this single inducible enzyme. We presume that the high level of expression of Xsc_{Ad} is representative for other organisms, which would explain the ease with which the inducible '63 kDa' monomer of Xsc can be detected by SDS/PAGE (cf. Figure 2) in the relevant α-, β- and δ-Proteobacteria as well as the Gram-positive organisms we tested (Table 2). The difference in sequence between subgroups 1 and 2, and between 1 and 3 (Figure 4), presumably explains why the *xsc* gene in *P. pantotrophus* NKNCYSA or *Desulfovibrio* sp. strain RZACYSA (Table 2) was not detected by the PCR primers we used. We wonder whether there is a subgroup of Xsc in each major group of bacteria (cf. Table 2), and possibly an entirely new group in e.g. *B. wadsworthia* (discussed in [1]).

The dendrogram (Figure 4) shows the relationship of Xsc to IlvB and IlvG, typical acetohydroxyacid synthases, and the clearly separate evolutionary lines. This relationship is clearly supported in Figure 3(A), where the activatory glutamate residue (Glu-47 in IlvG [51]) is seen to be conserved in Xsc. Similarly, the binding domain for the pyrophosphate moiety of ThDP is highly conserved (Figure 3B). Whereas the domain in subgroups 2 and 3 conforms with the PROSITE consensus pattern (L/I/V/M/F)(G/S/A)X_5PX_4(L/I/V/M/F/Y/W)X(L/I/V/M/F)XGD(G/S/A)(G/S/A/C), the domain in subgroup 1 (K_m^{app}, 2 μM; Table 1) shows that an extension of the consensus is needed to give (L/I/V/M/F)(G/S/A)X_5PX_4(L/I/V/M/F/Y/W)X(L/I/V/M/F/$\underline{Y}$)XGD(G/S/A)(G/S/A/C).

We now have information on the three catabolic reactions in the taurine degradative pathway in, e.g., *Alc. defragrans* (Scheme 1), so the major gap in our understanding is the lack of molecular data on transport, in contrast to the many ABC transporters in pathways for the assimilation of taurine sulphur (cf. [5,7,52]). The assimilation of taurine sulphur requires high affinity but low turnover [about 0.02 mkat/kg of protein in *Clostridium* sp. DSM 10612 (K. Denger, unpublished work)]; dissimilation requires high turnover (here 1.4 mkat/kg of protein, see above). The annotated sequence for *S. meliloti* has a putative ABC taurine transport system (*tauABC*$_{Sm}$) 2 kb upstream of the *ilvB-xsc*$_{Sm}$ gene (Figure 5) [53]; the incomplete genome of *B. fungorum* contains putative *tauABC*$_{Bf}$ adjacent to the *ilvB-xsc*$_{Bf}$ gene (Figure 5), and the contaminant in *L. major* has putative *tauABC*$_{Lm}$ 6 kb from the *xsc*$_{Lm}$ locus on the complementary strand (Figure 5). We hypothesize that the dissimilation of taurine (*S. meliloti*) or possibly sulphoacetaldehyde (*B. fungorum*) in these organisms is initiated after entering the cell through an ABC transporter.

Our understanding of regulation of the dissimilation of sulphonates is negligible. Some organisms seem to utilize only one sulphonate {*Alc. defragrans* [13] and *S. meliloti* (K. Denger, unpublished work and [6])}, whereas *Ralstonia* sp. strain EDS1

[2] and *B. fungorum* (K. Denger, unpublished work) utilize several sulphonates. *Ralstonia* sp. strain EDS1 seems to induce the expression of Xsc separately from the precursor reactions [2], a phenomenon also seen in *P. pantotrophus* (U. Rein and A.M. Cook, unpublished work). We now hypothesize a co-regulated set of contiguous genes for taurine degradation in *S. meliloti*. A potential regulator (SMb21 525) (cf. [53]) is located upstream of $tauABC_{Sm}$, on the complementary strand (Figure 5). Downstream of $tauABC_{Sm}$ (and an unidentified ORF) is a gene annotated *tauD*, an oxygenase (cf. [7]). However, our BLAST analyses revealed no similarity to oxygenases. The gene potentially encodes an oxidoreductase, possibly a taurine dehydrogenase. The neighbouring genes would encode Xsc_{Sm} and Pta_{Sm} (Figure 5). In contrast with degradation of the sole relevant substrate in one gene cluster, the hypothesis for *B. fungorum* is that several pathways converge at sulphoacetaldehyde, whose conversion into acetyl-CoA is induced by internal or external sulphoacetaldehyde. A potential regulator (contig 432, gene 1) is located upstream of $tauABC_{Bf}$, on the complementary strand (Figure 5). This gene has SMb21 525 as its closest homologue in BLAST searches in the NCBI database. The genes $ilvB\text{-}xsc_{Bf}$ and pta_{Bf}, downstream of $tauABC_{Bf}$, complete this gene cluster. If these testable hypotheses are confirmed, they will illustrate the power of a gene sequence to contribute to solving metabolic pathways discovered in other organisms. They also show how easily imprecise annotations give misleading impressions of gene function, especially when different branches of supergene families catalyse very different reactions.

We are grateful to K. Hollemeyer (University of the Saarland, Saarbrücken, Germany) for the MALDI-TOF MS determinations, to H. Erdbrink for first assaying the Xsc_{Ad}, to K. Bezares, C. Brüggemann, A. Günther, L. Khazoom and H. Liviatan for initial sequencing, to J.Ködding, Universität Konstanz, for the dynamic light-scattering experiments, and to J. Quinn (Queen's University, Belfast, N. Ireland, U.K.), J. M. Tiedje (University of Michigan, East Lansing, MI, U.S.A.), and S. Weidner and A. Pühler (University of Bielefeld, Bielefeld, Germany), for making bacteria available to us. We are also grateful to J. Quinn, D. Kelly (University of Warwick, Coventry, U.K.) and H. B. F. Dixon (University of Cambridge, Cambridge, U.K.) for valuable discussions. This work was supported by the European Union (SUITE: ENV4-CT98-0723) and the University of Konstanz.

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