Racemase activity effected by two dehydrogenases in sulfolactate degradation by *Chromohalobacter salexigens*: purification of (S)-sulfolactate dehydrogenase

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Chromohalobacter salexigens DSM 3043, whose genome has been sequenced, is known to degrade (R,S)-sulfolactate as a sole carbon and energy source for growth. Utilization of the compound(s) was shown to be quantitative, and an eight-gene cluster (Csal_1764–Csal_1771) was hypothesized to encode the enzymes in the degradative pathway. It comprised a transcriptional regulator (SuyR), a Tripartite Tricarboxylate Transporter-family uptake system for sulfolactate (SlcHFG), two sulfolactate dehydrogenases of opposite sulfonate stereochemistry, namely novel SlcC and ComC [(R)-sulfolactate dehydrogenase] [EC 1.1.1.272] and desulfonative sulfolactate sulfo-lyase (SuyAB) [EC 4.4.1.24]. Inducible reduction of 3-sulfo-pyruvate, inducible SuyAB activity and induction of an unknown protein were detected. Separation of the soluble proteins from induced cells on an anion-exchange column yielded four relevant fractions. Two different fractions reduced sulfo-pyruvate with NAD(P)H, a third yielded SuyAB activity, and the fourth contained the unknown protein. The latter was identified by peptide-mass fingerprinting as SlcH, the candidate periplasmic binding protein of the transport system. Separated SuyB was also identified by peptide-mass fingerprinting. ComC was partially purified and identified by peptide-mass fingerprinting. The (R)-sulfolactate that ComC produced from sulfo-pyruvate was a substrate for SuyAB, which showed that SuyAB is (R)-sulfolactate sulfo-lyase. SlcC was purified to homogeneity. This enzyme also formed sulfolactate from sulfo-pyruvate, but the latter enantiomer was not a substrate for SuyAB. SlcC was obviously (S)-sulfolactate dehydrogenase.

INTRODUCTION

3-Sulfolactate (hereafter sulfolactate) is a natural product in algae, animals, archaea, bacteria and plants (e.g. Bonsen *et al.*, 1969; Danko *et al.*, 2006; Graham & White, 2002; Lee & Benson, 1972; Roy *et al.*, 2003; Shibuya *et al.*, 1963; Weinstein & Griffith, 1988). The only major textbook to refer to sulfolactate (Metzler, 2003) implies a role in the biosynthesis of sulfoquinovose (SQ; 6-deoxy-6-sulfo-D-glucopyranose, the polar headgroup of the plant sulfolipid sulfoquinovosyl diacylglycerol), whereas, following Benning's work on SQ biosynthesis (Benning, 2007), sulfolactate is known to be an intermediate in the manipulation of SQ to a range of plant (and algal) products such as alkaloids (Folkers *et al.*, 1944), as suspected by Benson (Benson & Lee, 1972). The widespread occurrence of sulfolactate has apparently led to considerable diversity in the degradation of the compound,

and three desulfonative pathways are already known. Whereas sulfolactate can be desulfonated by sulfolactate sulfo-lyase (SuyAB) [EC 4.4.1.24] in *Paracoccus pantotrophus* NKNCYSA (Rein *et al.*, 2005), *Roseovarius nubinhibens* ISM has a bifurcated pathway (Denger *et al.*, 2009) which involves both sulfoacetaldehyde acetyltransferase [EC 2.3.3.15] (Ruff *et al.*, 2003) and (S)-cysteate sulfo-lyase (CuyA) [EC 4.4.1.25] (Denger *et al.*, 2006). The pathway to (S)-cysteate in *R. nubinhibens* is incomplete, because a sulfolactate racemase (or its equivalent) has not been identified.

The work with *R. nubinhibens* yielded the first candidate for a sulfolactate transporter, SlcHFG; the second SlcHFG (Csal_1767 – Csal_1769) was found in *Chromohalobacter salexigens* DSM 3043 associated with SuyAB (Denger *et al.*, 2009). The rarity of this transporter caused us to examine the relevant eight-gene cluster in *C. salexigens* (Csal_1764–Csal_1771) (Fig. 1). The genes encoding the presumed transporter are sandwiched between *suyAB* (Csal_1765–Csal_1766) and a pair of overlapping genes annotated to encode NAD(P)-coupled dehydrogenases (Csal_1770–Csal_1771). The latter is an orthologue of ComC, (R)-

Abbreviations: ComC, (R)-sulfolactate dehydrogenase; SlcC, (S)-sulfolactate dehydrogenase; SlcHFG, tripartite tricarboxylate transport (TTT)-family transporter for sulfolactate; SQ, sulfoquinovose; SuyAB, sulfolactate sulfo-lyase.

Fig. 1. Derived pathway of degradation of (R,S)-sulfolactate in *C. salexigens* DSM 3043 and the gene cluster encoding the pathway enzymes. The natures of the putative sulfite exporter and of the sulfite dehydrogenase are unknown.

sulfolactate dehydrogenase [EC 1.1.1.272] (Graupner *et al.*, 2000; Irimia *et al.*, 2004), so, requiring a racemase-equivalent, we hypothesized that CsaL_1770 represents (S)-sulfolactate dehydrogenase (SlcC). The current annotation of CsaL_1770 is SerA [(S)-3-phosphoglycerate dehydrogenase, EC 1.1.1.95] in serine biosynthesis. The eighth gene in the cluster is an orthologue of *suyR*, the putative transcriptional regulator of sulfolactate degradation in *P. pantotrophus* (Rein *et al.*, 2005). This information allowed a simple degradative pathway for (R,S)-sulfolactate to be drafted (Fig. 1), and the unknown stereospecificity of SuyAB (Rein *et al.*, 2005) to be deduced.

We now report supportive data for this pathway in *C. salexigens* DSM 3043. The paper concerns largely the initial scalar reaction in the pathway, that of novel (S)-sulfolactate dehydrogenase, which was purified and characterized.

METHODS

Chemicals. Racemic sulfolactate (Roy *et al.*, 2003) and 3-sulfolpyruvate (hereafter sulfopyruvate) (Denger *et al.*, 2001) were synthesized

as described in the papers cited. Initial work used the high quality sulfopyruvate (Denger *et al.*, 2001), but the new preparations were of low purity, which did not allow reliable quantification of e.g. K_m values at the end of the project. Commercial chemicals were of the highest purity available and purchased from Sigma-Aldrich, Roth, Merck, Biomol or Fluka.

Organisms, growth medium and growth conditions. *C. salexigens* DSM 3043, a marine gammaproteobacterium (Arahal *et al.*, 2001), was obtained from the German Culture Collection (DSMZ, Braunschweig, Germany). Cells were grown aerobically at 30 °C in Tris-buffered artificial seawater (Krejčík *et al.*, 2008) supplemented with 10 mM ammonium chloride and a seven-vitamin solution (Pfennig, 1978); the sole added source of carbon and energy was 10 mM (R,S)-sulfolactate or 20 mM acetate. Precultures (3 ml) were grown in 30 ml screw-cap tubes in a roller. Growth experiments were done on the 30 ml scale in 300 ml Erlenmeyer flasks shaken at 30 °C. Samples were taken at intervals to measure attenuation (580 nm), to assay protein, and to determine the concentrations of sulfolactate, sulfate or sulfite. Similar cultures were used to generate small amounts of cells for enzyme assays. Cultures (1 l) for protein purification were grown in 5 l Erlenmeyer flasks on a shaker. Cells were harvested at OD₅₈₀ 0.45 (about 110 mg protein l⁻¹) by centrifugation (15 000 g, 20 min, 4 °C), washed with 50 mM Tris buffer, pH 9.0, and stored frozen. The same buffer served as extraction buffer. Cell-free extracts free of nucleic acids after DNase

treatment (0.05 mg DNase I ml⁻¹) were generated after disruption by three passages through a French pressure cell set at 140 MPa (Junker *et al.*, 1994). The membrane/particulate fraction was sedimented by ultracentrifugation (220 000 g, 30 min, 4 °C) and the supernatant fluid was called the soluble fraction.

Cupriavidus necator JMP134 (DSM 4058), *Methylobacterium radiotolerans* (DSM 1819) and *Oligotropha carboxidovorans* (DSM 1227) were purchased from the DSMZ, and *Rhodococcus jostii* RHA1 was kindly supplied by W. W. Mohn (University of British Columbia, Vancouver, BC, Canada). Strains JMP134 and RHA1 were grown in our standard freshwater medium (Thurnheer *et al.*, 1986). Other organisms were grown in the salts medium recommended by DSMZ.

Enzyme assays. (S)-Sulfolactate dehydrogenase (SlcC) was routinely assayed photometrically (365 nm) at room temperature (about 23 °C) as the sulfo-pyruvate-dependent oxidation of NADH. The reaction mixture contained, in 25 mM Tris buffer, pH 9.0, sulfo-pyruvate (2 mM), NADH (0.25 mM) and protein (2–200 µg ml⁻¹), with which the reaction was started. Variants of the assay were used to determine kinetic constants. (R)-sulfolactate oxidoreductase (ComC) [EC 1.1.1.272] was routinely assayed photometrically (365 nm) at room temperature as the sulfolactate-dependent reduction of NADP⁺. The reaction mixture contained, in 0.5 M glycine/0.4 M hydrazine buffer, pH 9.0, sulfolactate (10 mM), NADP⁺ (2 mM) and protein (2–200 µg ml⁻¹), with which the reaction was started. 3-Phosphoglycerate dehydrogenase (SerA) [EC 1.1.1.95] was assayed under the same conditions, with 3-phosphoglycerate in place of sulfolactate. The reaction of each sulfolactate dehydrogenase could also be followed discontinuously in the presence of NADH or NADPH by ion chromatography by measuring the decrease (increase) of the sulfo-pyruvate (sulfolactate) concentration. In this case the concentration of sulfo-pyruvate was initially 0.5 mM. Sulfolactate sulfo-lyase (SuyAB) [EC 4.4.1.24] was assayed as release of sulfite from sulfolactate, with SuyAB from *P. pantotrophus* NKNCYSA as a positive control (Rein *et al.*, 2005). The MOPS buffer was replaced with 50 mM Tris buffer, pH 9.0. Sulfite dehydrogenase (SorAB) [EC 1.8.2.1] was assayed photometrically with K₃Fe(CN)₆ as electron acceptor (Reichenbecher *et al.*, 1999) as modified elsewhere (Denger *et al.*, 2008), and enzyme from *Cupriavidus necator* H16 served as positive control (Denger *et al.*, 2008).

Separation and purification of enzymes. Soluble fraction of *C. salexigens* in 25 mM Tris/sulfate buffer, pH 9.0 was loaded onto an anion-exchange chromatography column (Mono Q, HR 10/10, Pharmacia) at a flow rate of 1.0 ml min⁻¹. A step gradient of sodium sulfate up to 0.5 M was applied and all fractions were tested for SlcC, ComC and SuyAB.

Active fractions of SlcC or ComC were salted up to 1.7 M ammonium sulfate, the precipitate was spun off and the supernatant was subjected to hydrophobic interaction chromatography on Phenyl Superose HR 10/10 (Pharmacia). A linear decreasing gradient of ammonium sulfate in Tris/sulfate buffer, pH 8.5, was applied. Samples were centrifuged in Vivaspin concentrators (10 kDa cut-off, Sartorius). Gel filtration on Superose 12 (HR 10/30, Pharmacia) was done in 50 mM Tris/sulfate buffer, pH 9.0, including 150 mM sodium sulfate, at a flow rate of 0.4 ml min⁻¹. Standard high molecular mass proteins (aprotinin, RNase A, carbonic anhydrase, ovalbumin, conalbumin, aldolase and ferritin) served to calibrate the column.

Analytical methods. Protein in whole cells was quantified by a Lowry-type method (Kennedy & Fewson, 1968), omitting the initial acid treatment to avoid precipitation of salts originating from the medium. Soluble protein was assayed by protein-dye binding (Bradford, 1976). Denatured proteins were separated by 16% SDS-PAGE gels and stained with Coomassie brilliant blue R250 (Laemmli, 1970). Stained protein bands were cut out of the gel and subjected to

peptide-mass fingerprinting to identify the corresponding genes (done under contract at TopLab, Martinsried, Germany). Sulfolactate and sulfo-pyruvate were quantified by ion chromatography with suppression (Denger *et al.*, 2004). Sulfate release during growth was measured as the turbidity of a suspension of insoluble BaSO₄ (Sörbo, 1987) with at least a 1:5 dilution to avoid precipitation with salts of the medium. Sulfite was quantified as the fuchsin adduct (Denger *et al.*, 2001).

Various unsuccessful attempts were made to separate the enantiomers of sulfolactate by HPLC on a chiral column (Nucleosil Chiral-1 phase; Macherey-Nagel) according to the manufacturer's suggestions. Separations of the enantiomers of (R,S)-alanine and of (R,S)-lactate were achieved.

Software for sequence analysis. Analyses of the finished genome sequence (accession no. NC_007963, GenBank: CP000285) of *C. salexigens* DSM 3043 were done using the BLAST algorithm on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>) and the IMG page of JGI (<http://img.jgi.doe.gov/cgi-bin/pub/main.cgi>). The BLAST server on the Transport Classification Database (<http://www.tcdb.org/>), which is coupled to a predictor of transmembrane helices, was also used. Sequence data were manipulated with different subroutines from the LASERGENE program package (DNASTAR).

RESULTS

Growth of *C. salexigens* with (R,S)-sulfolactate

C. salexigens DSM 3043 utilized (R,S)-sulfolactate as sole source(s) of carbon and energy for growth. Sulfolactate was utilized quantitatively with stoichiometric release of the sulfonate moiety as sulfate; about 25 % of this sulfonate was released transiently as sulfite (Fig. 2 a, b). A molar growth yield of about 5 g protein (mol sulfolactate carbon)⁻¹ was observed, typical for the quantitative utilization of carbon (Cook, 1987); apparently both enantiomers of sulfolactate were completely oxidized. The growth rate was 0.12 h⁻¹ and a specific degradation rate for sulfolactate of 2.2 mkat (kg protein)⁻¹ could be calculated.

Activities of the hypothesized enzymes in the dissimilation of sulfolactate

The postulated pathway (Fig. 1) involved a three-component transport system for the polar sulfolactate, (S)-sulfolactate oxidoreductase (SlcC), (R)-sulfolactate oxidoreductase (ComC), desulfonative sulfolactate sulfo-lyase (SuyAB), an export system for sulfite and a putative sulfite dehydrogenase. The carbon skeleton would enter standard intermediary metabolism as pyruvate.

With crude extracts of sulfolactate-grown cells of *C. salexigens*, a specific enzyme activity of (S)-sulfolactate oxidoreductase (SlcC) of about 7.0 mkat (kg protein)⁻¹ was observed. With acetate-grown cells the activity was about 0.2 mkat (kg protein)⁻¹, which showed that the enzyme was inducible.

The specific activity of (R)-sulfolactate oxidoreductase (ComC) with crude extract of sulfolactate-grown cells was 0.8 mkat (kg protein)⁻¹. No activity was measurable with

Fig. 2. Growth of *C. salexigens* with (*R,S*)-sulfolactate as carbon source, showing transient accumulation of sulfite (a), and the behaviour of substrate(s) and product as a function of growth (b). ■, Protein; ○, sulfite; ●, sulfolactate; △, sulfate.

extracts of acetate-grown cells, suggesting inducibility of ComC.

Sulfolactate sulfo-lyase (SuyAB) could be detected in freshly prepared crude extract of sulfolactate-grown cells. The specific activity was 2.2 mkat (kg protein)⁻¹ in the crude extract and was found to be 3.3 mkat (kg protein)⁻¹ in the soluble fraction. No activity was detected in extracts of acetate-grown cells, so the enzyme activity was inducible. This enzyme was very unstable and was lost on freezing. Storage at 4 °C for 1 day resulted in a 50 % loss of activity.

Sulfite dehydrogenase (SorAB) could not be measured in crude extracts with either ferricyanide or eukaryotic cytochrome *c* as electron acceptor. This corresponded with the absence of appropriate gene candidates (*sorAB*) (Denger *et al.*, 2008).

Enrichment, purification or separation and identification of the relevant enzymes

Proteins in crude extracts of acetate-grown cells or of sulfolactate-grown cells were separated by SDS-PAGE. Two

strongly induced protein bands were observed (Fig. 3, lanes 1 and 2). The strong 43 kDa band (SuyB) was anticipated (Fig. 1) (Rein *et al.*, 2005) whereas the 30 kDa band was a novelty.

Proteins in the soluble extract of sulfolactate-grown cells were loaded on to an anion-exchange column. ComC and SlcC eluted in that order in slightly overlapping fractions at about 120 mM sulfate, and the 30 kDa band could be observed in the SlcC fraction (Fig. 3, lane 3). A broad activity peak of SuyAB with a very low specific activity eluted at about 170 mM sulfate.

The fraction with the highest activity of SlcC was subjected to hydrophobic interaction chromatography and the activity eluted after the end of the decreasing gradient. Gel filtration of SlcC followed as a further purification step, and to provide an estimate of the native molecular mass of the protein. SlcC was, thus, purified 91-fold to homogeneity with a recovery of 14 % (Table 1; Fig. 3, lane 5). The denatured enzyme had a molecular mass of about 34 kDa (Fig. 3). Native SlcC eluted from the gel filtration column with a retention time which indicated a molecular mass of about 61 kDa, suggesting a dimeric structure.

The MonoQ fraction with highest activity of ComC was also subjected to hydrophobic interaction chromatography. The enzyme eluted later than SlcC, and a 120-fold enrichment was attained. The corresponding protein band on SDS-PAGE indicated a molecular mass of 37 kDa (not shown).

Samples from the anion-exchanger with SuyAB activity were separated by SDS-PAGE. The prominent band at 43 kDa (SuyB; see Fig. 3) was enriched (not shown).

Fig. 3. Composite electropherograms (SDS-PAGE) of denatured proteins from different stages of the purification of SlcC from *C. salexigens*, with purified SlcH. Lane 1, crude extract of acetate-grown cells; lane 2, crude extract of sulfolactate-grown cells; lane 3, active fraction after anion-exchange chromatography; lane 4, active fraction after hydrophobic interaction chromatography; lane 5, purified enzyme (from a different preparation) after gel filtration chromatography; lanes 6 and 7, molecular mass markers (with masses in kDa); lane 8, the purified, unknown, induced protein later identified as SlcH.

Table 1. Purification of SlcC from *C. salexigens*

Purification step	Total protein (mg)	Total activity (nkat)	Recovery (%)	Specific activity [mkat (kg protein) ⁻¹]	Purification (-fold)
Crude extract	147	1088	100	7.4	1
Soluble fraction	104	884	81	8.5	1
Anion exchange	4.2	722	66	172	23
HIC*	0.9	266	24	310	42
Gel filtration	0.2	157	14	675	91

*HIC, hydrophobic interaction chromatography.

The unknown, inducible 30 kDa protein co-eluted from the anion-exchanger with SlcC (Fig. 3, lanes 2 and 3). The 30 kDa protein was essentially homogeneous in the following fraction (Fig. 3, lane 8).

The four separated proteins were excised from the SDS gels and subjected to peptide-mass fingerprinting. The data confirmed that the three enzymes did, indeed, represent the gene products of the candidate *slcC* gene (Csal_1770) (Fig. 1), the candidate *comC* gene (Csal_1771) and the candidate *suyB* gene (Csal_1766). The unknown protein was identified as Csal_1767, the candidate periplasmic binding protein (SlcH) of the sulfolactate transporter. We then compared separated (SDS-PAGE) membrane fractions of induced and non-induced cells, but there was no evidence for overexpressed membrane components (SlcFG) (not shown).

Some properties of the purified dehydrogenases

Both SlcC and ComC were stable at 4 °C for several weeks. Each could be frozen and thawed without loss of activity.

Purified SlcC converted sulfolactate into sulfolactate as demonstrated by ion chromatography; substrate and product were stable under the reaction conditions as shown by the controls without enzyme (Fig. 4). SlcC accepted NADH as co-substrate in this reaction. The K_m^{app} value for NADH was estimated as <50 μ M, that for sulfolactate as <140 μ M. Neither pyruvate nor oxaloacetate (5 mM) was a substrate, and neither compound was inhibitory.

SlcC could also be assayed in the forward direction, by trapping sulfolactate with hydrazine. Only NAD⁺ (and not NADP⁺) was a co-substrate. K_m^{app} values for NAD⁺ and (R,S)-sulfolactate were estimated as 2 mM and 7 mM, respectively. The specific activity under these conditions was only about 5 % of that in the reverse direction. The automatic annotation of *slcC* was '(S)-3-phosphoglycerate dehydrogenase'. We tested (S)-3-phosphoglycerate, (R)-malate, (R)-lactate, (S)-malate and (S)-lactate and none was a substrate for SlcC (and none was inhibitory). We compared the sequences of 14 candidate SlcCs (see below) with those of characterized 3-phosphoglycerate dehydrogenases [EC 1.1.1.95] in a dendrogram, and found that they

clustered separately (not shown). SlcC is an enzyme in its own right.

ComC also converted sulfolactate into sulfolactate (data not shown). Neither pyruvate nor oxaloacetate (5 mM) was a substrate. NADPH served as co-substrate. The K_m^{app} value for NADPH was about 100 μ M, that for sulfolactate about 140 μ M. In the assay in the oxidative direction with hydrazine buffer, NADP⁺ could not be replaced by NAD⁺. Neither (S)-malate, (S)-lactate, (R)-malate nor (R)-lactate was a substrate for ComC (and none inhibited the enzyme). K_m^{app} values of 1.2 ± 0.02 mM for NADP⁺ and 19 ± 5 mM for sulfolactate were calculated.

Coupling SlcC or ComC to SuyAB

We were able to separate the enantiomers of alanine and lactate on a commercially available HPLC column, but we found no conditions which separated the enantiomers of (R,S)-sulfolactate. So we deduced the stereochemistry involved from the thorough work done elsewhere with ComC (Graham & White, 2002; Graupner *et al.*, 2000).

Sulfolactate, NADH and SlcC were incubated for 15 min, after which separated SuyAB was added. There

Fig. 4. Conversion of 0.5 mM sulfolactate (▲) to sulfolactate (■) by SlcC. In control experiments, sulfolactate (△) or sulfolactate (□) was stable in the absence of enzyme.

was negligible formation of sulfite. In contrast, after preincubation of sulfoxyruvate, NADPH and ComC, addition of SuyAB led to release of significant amounts of sulfite. ComC generates (R)-sulfolactate from sulfoxyruvate (Graupner *et al.*, 2000), so we deduce that (R)-sulfolactate is the substrate for SuyAB. Correspondingly, the sulfolactate, which is generated by SlcC from sulfoxyruvate (Fig. 4) and which is not a substrate for SuyAB, must be (S)-sulfolactate.

Bioinformatic data

There are currently 13 genomes with a *suyAB-slcC-comC* cluster (Table 2), and several more with *slcC* or *comC* elsewhere on the chromosome. Representative organisms from Table 2 were tested for growth with sulfolactate. *Methylobacterium radiotolerans* and *Oligotropha carboxidovorans* grew with the compound and excreted sulfate, whereas *Cupriavidus necator* and *Rhodococcus jostii* did not grow.

DISCUSSION

Growth of *C. salexigens* with sulfolactate, detected elsewhere (Denger *et al.*, 2009), is seen to be quantitative (Fig. 2a, b). The release of sulfite into the medium (Fig. 2a) indicates the presence of an unidentified sulfite exporter in the cytoplasmic membrane. The unidentified sulfite dehydrogenase, which we place in the periplasm (Denger *et al.*, 2008; Kappler *et al.*, 2000), completes the pathway (Fig. 1).

The pathway is inducible (e.g. Fig. 3). No regulatory work has been done, but the putative GntR-type transcriptional regulator, SuyR, first discovered contiguous with *suyAB* in *Paracoccus denitrificans* NKNCYSA (Rein *et al.*, 2005), is also found in all organisms in Table 2. So the hypothesis for the role of SuyR is still valid.

The initial step in the pathway is an uptake system, SlcHFG [TC 2.A.80.--] (Fig. 1). When first proposed in *R. nubinhibens* (Denger *et al.*, 2009), the experimental support for SlcHFG was from reverse-transcription PCR. We now have strong expression of one component, SlcH (Fig. 3), as proof that at least one of the genes is transcribed. However, we still do not know exactly what is transported. Is only one enantiomer of (R,S)-sulfolactate transported, or both? If the former, there must be a second transporter, whose identity is unknown.

SlcHFG is a rare transporter (Denger *et al.*, 2009), being found in organisms in Table 2 only in *C. salexigens*, *Fulvimarina pelagi* (some five to seven genes upstream) and possibly in *Oligotropha carboxidovorans*. Alternative uptake mechanisms seem to be DAACS and ABC transporters (Table 2). Many organisms seem to have no transporter for sulfolactate (Table 2), and sulfolactate does not support growth (see Results). In the latter cases, we presume that external sulfolactate is not utilized (e.g. *Rhodococcus jostii* RHA1), because a transport system is not available, and that any sulfolactate in the cell is generated intracellularly from a precursor, analogous to the situation in *Ruegeria pomeroyi* DSS-3, where the sulfolactate is apparently

Table 2. Organisms containing the *suyAB* and *slcC-comC* cluster

Only *C. salexigens* has the *slcHFG* genes in this cluster; *F. pelagi* has the genes for this transporter elsewhere on the genome, and most organisms encode no transporter at this position. All organisms encode a putative transcriptional regulator (SuyR) at one or other end of the cluster. The acronyms 'DAACS' and 'ABC transporter' represent the dicarboxylate/amino acid:cation symporter family [TC 2.A.23.--] and the ATP binding cassette superfamily [TC 3.A.1.--].

Organism	Tag	<i>suyR</i>	<i>suyA</i>	<i>suyB</i>	Genes between <i>suyB</i> and <i>slcC</i>	<i>slcC</i>	<i>comC</i>
<i>Chromohalobacter salexigens</i> DSM 3043	CsaI_	1764	1765	1766	<i>slcHFG</i>	1770	1771
<i>Cupriavidus necator</i> JMP134	Reut_	B3533	B3528	B3529	None	B3530	B3531
<i>Cupriavidus taiwanensis</i> LMG 19424	RALTA_	B0844	B0839	B0840	None	B0841	B0842
<i>Fulvimarina pelagi</i> HTCC 2506	FP2506_1	14334	14329	14324	None	14319	14314
<i>Hoeflea phototrophica</i> DFL42	HPDFL43_	17106	17101	17096	None	17091	17086
<i>Herminiimonas arsenicoxydans</i>	HEAR	3334	3336	3337	Encode an ABC transporter?	3340	3341
<i>Meiothermus ruber</i>	MrubDRAFT_	06820	06830	06840	None	06850	06860
<i>Methylobacterium radiotolerans</i> DSM 1819	Mrad2831_	5975	5980	5979	Encode a DAACS transporter	5977	5976
<i>Oligotropha carboxidovorans</i> OM5	OCAR_	7559	7558	7557	Variant <i>slcHFG</i>	7552	7551
<i>Rhodococcus jostii</i> RHA1	RHA1_ro	08849	08848	08847	No transport genes	08844	08843
<i>Variovorax paradoxus</i> S110	Vapar_	1590	3818	3819	None	3820	3821
<i>Verminephrobacter eiseniae</i>	Veis_	0894	0893	0892	None	0891	0890
<i>Bordetella petrii</i>	Bpet	0416	0415	0414	Encode an ABC transporter	0409	0408

generated from 2,3-dihydroxypropanesulfonate (Denger *et al.*, 2009).

In contrast to the incomplete story (stories) at the cell surface, there is clarity about the reactions of the scalar enzymes in the pathway. The novelty is the (S)-sulfolactate dehydrogenase (SlcC) (Fig. 1). Its function is to convert the (S)-enantiomer into sulfo-pyruvate, which allows ComC to convert sulfo-pyruvate into (R)-sulfolactate, the substrate for the desulfonation reaction, SuyAB. The novel enzyme belongs to [EC 1.1.1.-] in the Enzyme List, and we suggest the systematic name '(S)-sulfolactate:NAD⁺ oxidoreductase'. The accepted name could be (S)-sulfolactate dehydrogenase.

There are, thus, at least three sulfolactate oxidoreductases. The best-characterized is ComC [EC 1.1.1.272] in the biosynthesis of coenzyme M in *Methanococcus jannaschii* (and methanogens in general) (Graham & White, 2002; Graupner *et al.*, 2000) and in spore-formers. The least-understood is SlcD [EC 1.1.99.-], a membrane-bound enzyme in the degradation of sulfolactate in *R. nubinihibens* ISM (Denger *et al.*, 2009). Finally, there is SlcC, with its unusual role as a partial racemase in a degradative pathway, which is introduced here.

The observation of two dehydrogenases effecting racemization seems to be novel. Others have used the hypothesis that this could occur, but their experimental data showed that a different metabolic route was used (Haupt & Bocker, 1965; Lincoln *et al.*, 1987).

There are about 50 orthologues of *suyAB* in the open databases. Only about 30 % of these orthologues (Table 2) are in organisms which seem to involve racemization via SlcC and ComC as in Fig. 1. Other orthologues (30 %) are found in thermophilic archaea, all of which have a potential racemase encoded adjacent to *suyB*. The other orthologues of *suyAB* are found in several different clusters (not shown). The diversity in pathways of sulfonate degradation (e.g. Baldock *et al.*, 2007; Denger *et al.*, 2009; Weinitschke *et al.*, 2010) is, thus, recurrent, and indicates how widespread the sulfonates must be in marine and terrestrial environments.

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