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Bifurcated Degradative Pathway of 3-Sulfolactate in *Roseovarius nubinhibens* ISM via Sulfoacetaldehyde Acetyltransferase and (*S*)-Cysteate Sulfolyase▽†

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Data from the genome sequence of the aerobic, marine bacterium *Roseovarius nubinhibens* ISM were interpreted such that 3-sulfolactate would be degraded as a sole source of carbon and energy for growth via a novel bifurcated pathway including two known desulfonative enzymes, sulfoacetaldehyde acetyltransferase (EC 2.3.3.15) (Xsc) and cysteate sulfo-lyase (EC 4.4.1.25) (CuyA). Strain ISM utilized sulfolactate quantitatively with stoichiometric excretion of the sulfonate sulfur as sulfate. A combination of enzyme assays, analytical chemistry, enzyme purification, peptide mass fingerprinting, and reverse transcription-PCR data supported the presence of an inducible, tripartite sulfolactate uptake system (SlcHFG), and a membrane-bound sulfolactate dehydrogenase (SlcD) which generated 3-sulfopyruvate, the point of bifurcation. 3-Sulfopyruvate was in part decarboxylated by 3-sulfopyruvate decarboxylase (EC 4.1.1.79) (ComDE), which was purified. The sulfoacetaldehyde that was formed was desulfonated by Xsc, which was identified, and the acetyl phosphate was converted to acetyl-coenzyme A by phosphate acetyltransferase (Pta). The other portion of the 3-sulfopyruvate was transaminated to (*S*)-cysteate, which was desulfonated by CuyA, which was identified. The sulfite that was formed was presumably exported by CuyZ (TC 9.B.7.1.1 in the transport classification system), and a periplasmic sulfite dehydrogenase is presumed. Bioinformatic analyses indicated that transporter SlcHFG is rare but that SlcD is involved in three different combinations of pathways, the bifurcated pathway shown here, via CuyA alone, and via Xsc alone. This novel pathway involves ComDE in biodegradation, whereas it was discovered in the biosynthesis of coenzyme M. The different pathways of desulfonation of sulfolactate presumably represent final steps in the biodegradation of sulfoquinovose (and exudates derived from it) in marine and aquatic environments.

Sulfolactate (Fig. 1A) is a widespread natural product, which contains the stable C-SO_3^- bond. The compound is known to be (i) a component (5% of dry weight) of bacterial endospores (5), (ii) an intermediate in the biosynthesis of coenzyme M in archaea (55), (iii) in equilibrium with (*S*)-cysteate in mammals (54), (iv) involved in the metabolism of sulfoquinovose (6-deoxy-6-sulfo-D-glucopyranose, the polar moiety of the plant sulfolipid) in plants and algae (e.g., see reference 48), and (v) an intermediate in the bacterial degradation of sulfoquinovose (44).

Research on the biodegradation of organosulfonates has concentrated on compounds containing one to four carbon atoms (C_1, C_2, C_3, or C_4 sulfonates), because where appropriate, larger molecules all seemed to be processed via one of the five desulfonative reactions that have been elucidated. Pathways from (i) sulfoquinovose yield, e.g., sulfoacetate or sulfolactate and 2,3-dihydroxy-1-sulfopropane (36, 44), (ii) taurocholate and *N*-acetyltaurine yield taurine (37, 43), and (iii) *N*-methyltaurine yield sulfoacetaldehyde (52). The five desulfonation reactions are two oxygenolyses for the C_1 and C_4 sulfonates (30, 39), phosphatolysis of sulfoacetaldehyde by sulfoacetaldehyde acetyltransferase (Xsc) (EC 2.3.3.15) (8, 45), dehydratase-like elimination of sulfite from sulfolactate (sulfolactate sulfo-lyase SuyAB) (EC 4.4.1.24) (42), and the pyridoxal phosphate chemistry involved in (*S*)-cysteate sulfo-lyase (CuyA) (EC 4.4.1.25) (9, 14). Although not relevant here, the range of known desulfonation reactions is wider when enzymes involved in the assimilation of sulfonate-sulfur are considered (e.g., see references 17, 27, and 32).

Whereas sulfoacetaldehyde and sulfolactate are described in the preceding paragraph as degradative intermediates in different, independent pathways, they are also known as biosynthetic intermediates in one pathway, the formation of coenzyme M (55), during which (*R*)-3-sulfolactate is oxidized to sulfopyruvate by (*R*)-2-hydroxyacid dehydrogenase (ComC) (EC 1.1.1.272). The latter compound is decarboxylated to sulfoacetaldehyde by sulfopyruvate decarboxylase (EC 4.1.1.79) (ComDE) (55). Bioinformatic analyses of the genome of the bacterium *Roseovarius nubinhibens* ISM revealed the presence of gene candidates to encode ComDE, Xsc, and CuyA, which, with other gene products, allowed a novel, bifurcated degradative pathway for sulfolactate to be proposed (Fig. 1). A possible alternative pathway via SuyAB was considered to be unlikely, because no candidate *suyAB* genes were detected on the genome.

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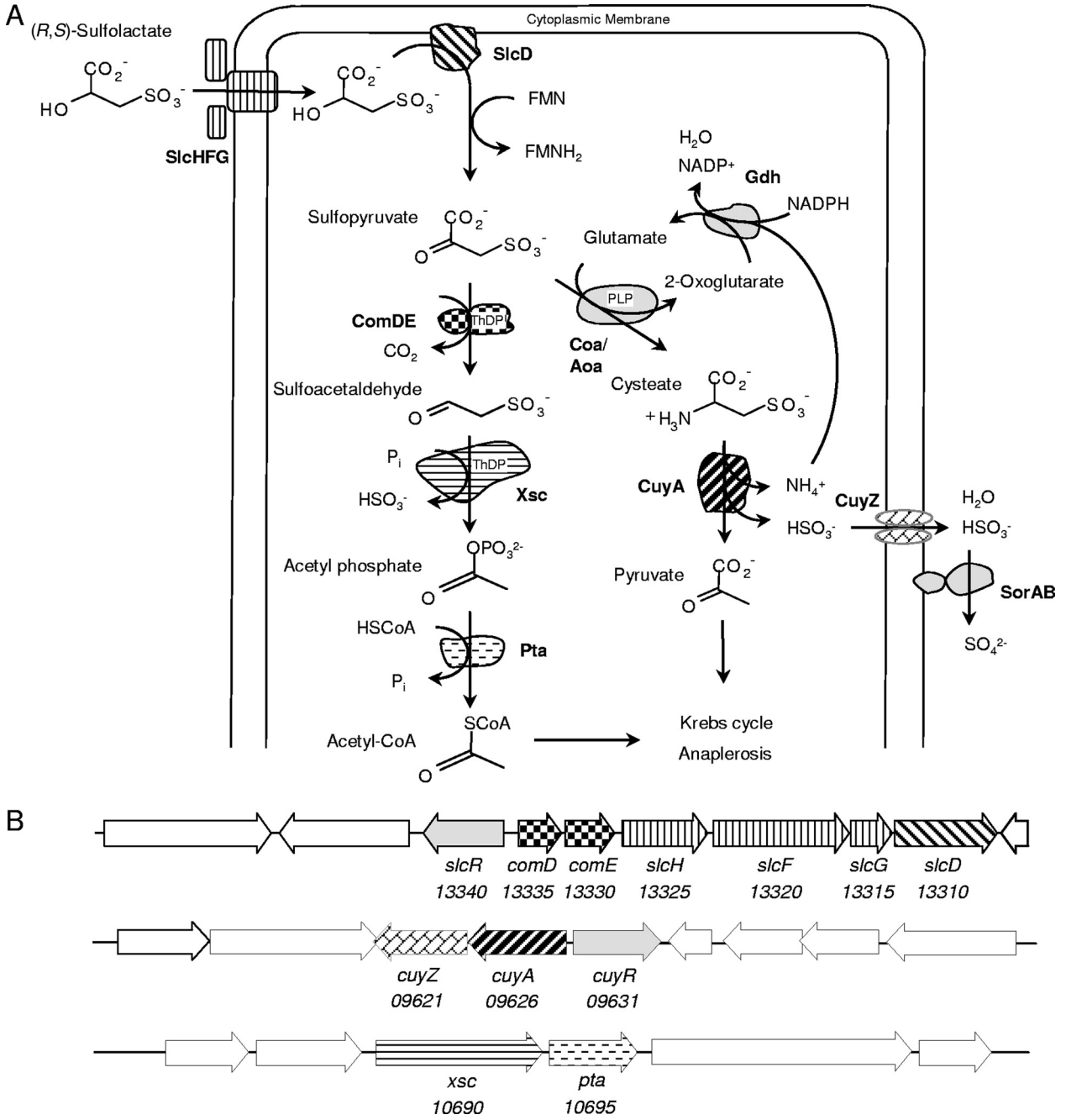

FIG. 1. Hypothesized bifurcated degradative pathway of sulfolactate in *R. nubinhibens* ISM (A) and the corresponding gene clusters (B), with our annotation of the locus tags. Abbreviations of enzyme names are in the main text. ThDP, thiamine diphosphate; PLP, pyridoxal-5-phosphate; CoA, coenzyme A. Taurine has several degradative pathways, but that in *Ruegeria pomeroyi* DSS-3 (21) corresponds to that found in strain ISM.

We now report that *R. nubinhibens* ISM utilizes sulfolactate inducibly as a sole source of carbon and energy for growth. There is extensive experimental support for the bifurcated pathway shown in Fig. 1A.

MATERIALS AND METHODS

Chemicals. Racemic sulfolactate was synthesized as described elsewhere (44), as were sulfopyruvate and the bisulfite addition complex of sulfoacetaldehyde (13). Commercial chemicals were of the highest purity available and purchased from Sigma-Aldrich, Roth, Merck, Biomol, or Fluka. *Taq* DNA polymerase, Moloney murine leukemia virus reverse transcriptase, and RNase-free DNase were from MBI-Fermentas.

Organisms, growth, harvesting of cells, and preparation of cell extracts. *R. nubinhibens* ISM, a marine alphaproteobacterium (19), was kindly made available by M. A. Moran (Georgia, United States). Cells were grown aerobically at 30°C in a modified *Silicibacter* basal medium (14) containing 0.05% yeast extract; the sole added source of carbon and energy was 10 mM (*R*,*S*)-sulfolactate or (*S*)-cysteate, 20 mM taurine, or 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; 6 mM sulfolactate was used. Samples were taken at intervals to measure optical density (580 nm), to assay protein, and to determine the concentration of sulfolactate, sulfate, or sulfite. Similar cultures

were used to generate small amounts of cells for enzyme assays. Cultures (1 liter) for protein purification were grown in 5-liter Erlenmeyer flasks on a shaker. Cells were harvested at an optical density at 580 nm of 0.5 (about 170 mg protein $liter^{-1}$) by centrifugation (15,000 × *g*, 20 min, 4°C); washed with 50 mM potassium phosphate buffer, pH 7.5 (containing 5 mM $MgCl_2$); and stored frozen. The same buffer served as extraction buffer. Cell extracts free of nucleic acids (0.05 mg DNase I ml^{-1}) were generated by disruption via four passages through a French pressure cell set at 140 MPa (26) or by ultrasonication. The membrane/particulate fraction was sedimented by ultracentrifugation (220,000 × *g*, 30 min, 4°C), and the supernatant fluid was called the soluble fraction. Solubilization of membranes was done by stirring with Triton X-100 (0.5 mg [mg protein]$^{-1}$) on ice. After ultracentrifugation (220,000 × *g*, 30 min, 4°C), the clear supernatant fluid was called the membrane fraction.

Chromohalobacter salexigens DSM 3043 was obtained from the German Culture Collection (Braunschweig, Germany). *Roseobacter* sp. strain MED193 (http://www.roseobase.org/roseo/med193.html) was kindly supplied by J. Pinhassi (University of Kalmar, Kalmar, Sweden). *Roseovarius* sp. strain 217 (46) was kindly made available by J. C. Murrell (University of Warwick, Coventry, United Kingdom). These three organisms were grown in Tris-buffered artificial seawater (33). *Ruegeria* (*Silicibacter*) *pomeroyi* DSS-3 (19, 58) was kindly provided by M. A. Moran and grown in modified *Silicibacter* basal medium.

Enzyme assays. Sulfoacetaldehyde acetyltransferase (Xsc, EC 2.3.3.15) was assayed as the formation of sulfite or acetylphosphate from sulfoacetaldehyde (45). (*S*)-Cysteate sulfo-lyase (CuyA, EC 4.4.1.25) was assayed as the release of sulfite from (*S*)-cysteate (14). 3-Sulfolactate sulfo-lyase (SuyAB, EC 4.4.1.24) was assayed as the release of sulfite from sulfolactate; an extract of *Paracoccus pantotrophus* NKNCYSA (42) was used as a positive control. The photometric assay for sulfolactate dehydrogenase (SlcD) contained 50 mM Tris-HCl (pH 8 or pH 9), 20 mM sulfolactate, 1 mM ferricyanide (ε_{420} = 0.9 mM^{-1} cm^{-1}), or 0.1 mM dichlorophenol indophenol (ε_{600} = 16.1 mM^{-1} cm^{-1}) and 0.05 to 0.5 mg protein ml^{-1}. To demonstrate substrate disappearance and product formation by ion chromatography, the assay consisted of 0.5 mM sulfolactate, 5 mM ferricyanide, and membrane fraction (0.6 mg protein ml^{-1}) in 50 mM Tris-HCl, pH 8.0. The assay for cysteate:2-oxoglutarate aminotransferase (Coa, EC 2.6.1.-) was adapted from that used by Mikosch et al. (38), and the formation of glutamate was measured discontinuously by high-pressure liquid chromatography (HPLC) after derivatization. Glutamate dehydrogenase (Gdh, EC 1.4.1.4) was assayed photometrically (47). The assay for sulfopyruvate decarboxylase (ComDE; EC 4.1.1.79) was contained in 50 mM Tris-HCl (pH 7.5), 1 mM thiamine diphosphate, 1 to 5 mM sulfopyruvate, and protein (0.1 to 1 mg ml^{-1}). Routinely the disappearance of sulfopyruvate was followed by ion chromatography. Occasionally the formation of sulfoacetaldehyde was measured by HPLC after derivatization. Phosphate acetyltransferase (Pta, EC 2.3.1.8) was assayed photometrically in fresh extracts according to standard methods (4). Sulfite dehydrogenase (Sor, EC 1.8.2.1) was assayed photometrically with $K_3Fe(CN)_6$ as an electron acceptor (41), with modifications that are described elsewhere (16). Taurine:pyruvate aminotransferase (Tpa, EC 2.6.1.77) was assayed discontinuously by HPLC for alanine formation after derivatization (53). Simultaneous operation of both branches of the bifurcated pathway was explored with 3-sulfopyruvate as a substrate in crude extract of sulfolactate-grown cells at 37°C; the reaction mixture contained 50 mM Tris-HCl buffer (pH 8.0), 10 mM sulfopyruvate, and 5 mM glutamate, and samples were taken at zero time and 15 min to determine the concentrations of cysteate and sulfoacetaldehyde.

Separation and purification of enzymes. The soluble fraction of *R. nubinhibens* ISM in 50 mM potassium phosphate buffer, pH 7.2, 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 active separated fractions of CuyA, Xsc, and ComDE were eluted.

Active fractions of CuyA were pooled, rebuffered in 50 mM Tris-sulfate (pH 9.0) on PD10 columns (Sephadex G-25; GE Healthcare, München, Germany), and subjected to a second anion-exchange chromatography step at pH 9.0 (see above). The same increasing sodium sulfate gradient was applied, and CuyA eluted at about 100 mM sodium sulfate.

For purification of ComDE, the soluble fraction was brought to 1.7 M ammonium sulfate, the precipitate was spun off, and the supernatant was subject to hydrophobic interaction chromatography on Phenyl Superose HR 10/10 (Pharmacia). A linear decreasing gradient of ammonium sulfate in potassium phosphate buffer, pH 7.2, was applied, and ComDE eluted at 0 mM ammonium sulfate. The rebuffered active fraction was loaded onto the anion-exchange chromatography column as a second purification step, and ComDE eluted at about 250 mM sodium sulfate. Active fractions were concentrated using Vivaspin units (10-kDa cutoff, PES membrane; Sartorius, Göttingen, Germany). Gel filtration was used as a third purification step (Superose 12 HR 10/30; Pharmacia) in 50 mM potassium phosphate buffer, pH 7.2, including 150 mM sodium sulfate at a flow rate of 0.4 ml min^{-1}. Standard high-molecular-weight proteins (conalbumin, aldolase, catalase, and ferritin) were used to generate a calibration curve, and the molecular weight of native ComDE was estimated by interpolation.

To separate SlcD, the solubilized membrane fraction was loaded onto an anion-exchange chromatography column with the buffers described above, including 0.1% (vol/vol) Triton X-100, or onto a gel filtration column with the buffer system described above, including 0.1% (vol/vol) Triton X-100 at a flow rate of 0.2 ml min^{-1}.

Analytical methods. Protein in whole cells was quantified by a Lowry-type method (31) without the initial acid treatment to avoid precipitation of salts originating from the medium. Soluble protein was assayed by protein dye binding (6). Denatured proteins were separated by 16% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and stained with Coomassie-brillant blue R250 (34). Stained protein bands were cut out of the gel and subjected to peptide mass fingerprinting to identify the corresponding genes, which was done under contract at TopLab (Martinsried, Germany). Sulfolactate, sulfopyruvate, and isethionate were quantified by ion chromatography with suppression (15, 50). Sulfate release during growth was measured as turbidity of a suspension of insoluble $BaSO_4$ (49); a 1:10 dilution of the sample was necessary to avoid a precipitate with other components of the medium. Sulfite was quantified as the fuchsin adduct (12). Acetylphosphate was determined chemically as iron(III) acetyl hydroxamate (40). Reversed-phase HPLC was used to quantify derivatized alanine, glutamate, or cysteate (35) or derivatized sulfoacetaldehyde (11). Reverse transcription-PCR (RT-PCR) was done as detailed elsewhere (33) using the primer pairs shown in Table S1 in the supplemental material. The RNA was tested prior to use for residual DNA with primer pair ISM-pta-F and ISM-pta-R. Chromosomal DNA from *R. nubinhibens* ISM was used as a positive control for PCRs.

Bioinformatic analyses. Analysis of the draft genome sequence (accession no. NZ_AALY00000000) of *R. nubinhibens* ISM was done using the BLAST algorithm on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). 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 up to 2 April 2009 were used. Sequence data were manipulated with different subroutines from the LASERGENE program package (DNAStar, Madison, WI). Alignments were made using ClustalX and plotted in NJPlot (51). Primers for RT-PCR and PCR were designed using the program Amplify (version 1.2).

RESULTS

Growth of *R. nubinhibens* ISM in minimal medium. Given the genome sequence of *R. nubinhibens* ISM, we postulated the utilization of at least three organosulfonates as sole sources of carbon and energy for growth. Preliminary experiments in minimal medium gave nonreproducible growth, but the supplement of yeast extract solved the problem. Strain ISM then grew reproducibly with (*R*,*S*)-sulfolactate, (*S*)-cysteate, taurine, or acetate as the sole source of carbon and energy, with similar molar growth yields (about 6 g protein [mol C]$^{-1}$), typical for the quantitative utilization of carbon (7). In all appropriate cases, quantitative recovery of the sulfonate moiety as sulfate was observed. The growth rate (μ) with 0.05% yeast extract alone was about 0.23 h^{-1}, and about 0.3 mM sulfate was excreted (not shown). When sulfolactate was present, the growth rate was initially about 0.23 h^{-1}, at which a low release of sulfate was observed, although no significant disappearance of sulfolactate was detected (Fig. 2). The growth rate then dropped to 0.10 h^{-1}, and both substrate disappearance and sulfate release were concomitant with growth and quantitative (Fig. 2). Under these last conditions, the growth yield was 6 g protein (mol C)$^{-1}$, so with the growth yield, a specific turnover rate of 1.4 mkat (kg protein)$^{-1}$ for sulfolactate could be calculated. We attributed the initial rapid growth largely to the utilization of organic components in the yeast extract, while the

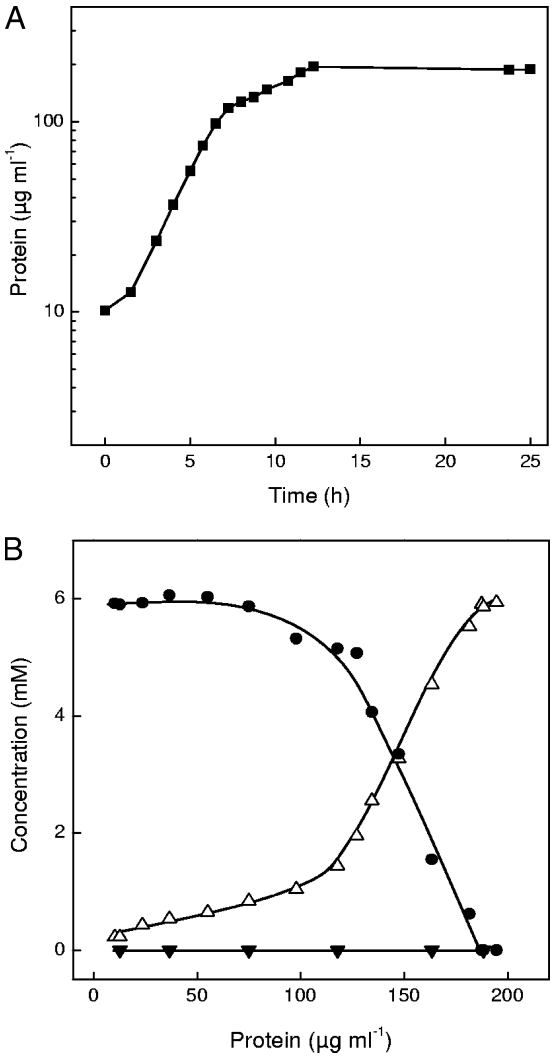

FIG. 2. Growth of *R. nubinhibens* ISM with sulfolactate as the carbon source (A) and changes in concentrations of substrate and product as a function of growth (B). ●, sulfolactate; △, sulfate; ▼, sulfite.

slower growth (after 6 h) obviously involved the utilization of both enantiomers of sulfolactate.

C. salexigens DSM 3043 utilized sulfolactate quantitatively with stoichiometric release of sulfate. We confirmed that *Roseovarius* sp. strain 217 (2) and *R. pomeroyi* DSS-3 (14) utilized (*S*)-cysteate quantitatively, but neither organism utilized sulfolactate extensively (i.e., ~10%). Strains 217 and DSS-3 involve Xsc in taurine metabolism, as observed previously (2, 21). *Roseobacter* sp. strain MED193 did not grow with sulfolactate but did grow quantitatively with taurine as the sole source of carbon and energy.

Induction of desulfonative and other enzymes. *R. nubinhibens* ISM was grown with (*R*,*S*)-sulfolactate, (*S*)-cysteate, taurine, or acetate as the sole source of carbon and energy; the cells were harvested and disrupted; and cell extracts were prepared. A set of 10 enzymes in the soluble and particulate fractions of these extracts was assayed (Table 1). No activity of Xsc or CuyA, each a desulfonative enzyme, was detected with extracts of acetate-grown cells, whereas Xsc was found in extracts of taurine-grown cells and of sulfolactate-grown cells, and CuyA was found in extracts of cysteate-grown cells and of sulfolactate-grown cells. Xsc and CuyA were thus inducible. Further, no activity of SuyAB was detected (Table 1).

The postulated bifurcated pathway to degrade sulfolactate involves a sulfolactate dehydrogenase to generate sulfopyruvate (Fig. 1A). *S*ulfo*lac*tate *d*ehydrogenase (SlcD) activity, which was dependent on an artificial electron acceptor (ferricyanide), was detected with sulfolactate-grown cells only (Table 1). The enzyme is thus inducible. The reaction observed was a conversion of sulfolactate to sulfopyruvate (Fig. 3A). There was no sulfolactate dehydrogenase activity with $NADP^+$ (i.e., orthologous to the ComC of *Methanocaldococcus jannaschii* [MJ1425] [24]), NAD^+, flavin adenine dinucleotide, flavine mononucleotide (FMN), or cytochrome *c*.

SlcD was found in both the soluble and the particulate fractions, but the specific activity in the particulate fraction (0.8 mkat [kg protein]$^{-1}$; Table 1) was fivefold higher than that in the soluble fraction, so the enzyme was considered to be membrane associated. SlcD was attributed to the (membrane-bound) 2-hydroxyacid dehydrogenases (see below); there was no activity with (*R*,*S*)-mandelate or (*R*,*S*)-malate, but (*S*)-lactate was oxidized with about 10% of the rate with sulfolactate.

The CuyA branch of the pathway to degrade sulfopyruvate requires a cysteate transaminase. A constitutive (*S*)-cysteate: 2-oxoglutarate aminotransferase (Coa) in bacteria is known (38), and this enzyme was found to be constitutive in *R. nubinhibens* ISM (Table 1). Correspondingly, a constitutive glutamate dehydrogenase (Gdh) was observed, and it recycled the amino group to generate (*S*)-cysteate from sulfopyruvate (Fig. 1A; Table 1). No pyruvate-coupled (*S*)-cysteate transaminase was detected.

The Xsc branch of the pathway requires activity of the sulfopyruvate decarboxylase (ComDE) noted in the introduction (Fig. 1A). Activity of ComDE could be detected as substrate disappearance in extracts of sulfolactate-grown cells only (Table 1), so the enzyme was considered to be inducible.

Phosphate acetyltransferase (Pta) is usually essential in the degradation of sulfoacetaldehyde (Fig. 1A) (8; see also reference 2). Inducible enzyme activity was detected (Table 1).

TABLE 1. Specific activities of enzymes in crude extracts of *R. nubinhibens* ISM grown with different sole sources of carbon and energy

Enzyme	Specific enzyme activity (mkat [kg protein]$^{-1}$) in extracts from cells grown with:			
	Sulfolactate	Taurine	Cysteate	Acetate
Xsc	0.9	1.5	0.1	BLD[a]
CuyA	1.0	BLD	5.2	BLD
SuyAB	BLD	BLD	BLD	BLD
SlcD[b]	0.8	0.1	0.1	0.1
Coa	1.2	1.0	0.8	1.3
Gdh	0.4	0.5	0.5	0.4
ComDE	0.9	BLD	BLD	BLD
Pta	6.9	0.5	BLD	BLD
Sor	0.4	0.4	0.3	0.2
Tpa	0.5	4.9	0.2	0.1

[a] BLD, below the limit of detection.
[b] Activities in the particulate fraction.

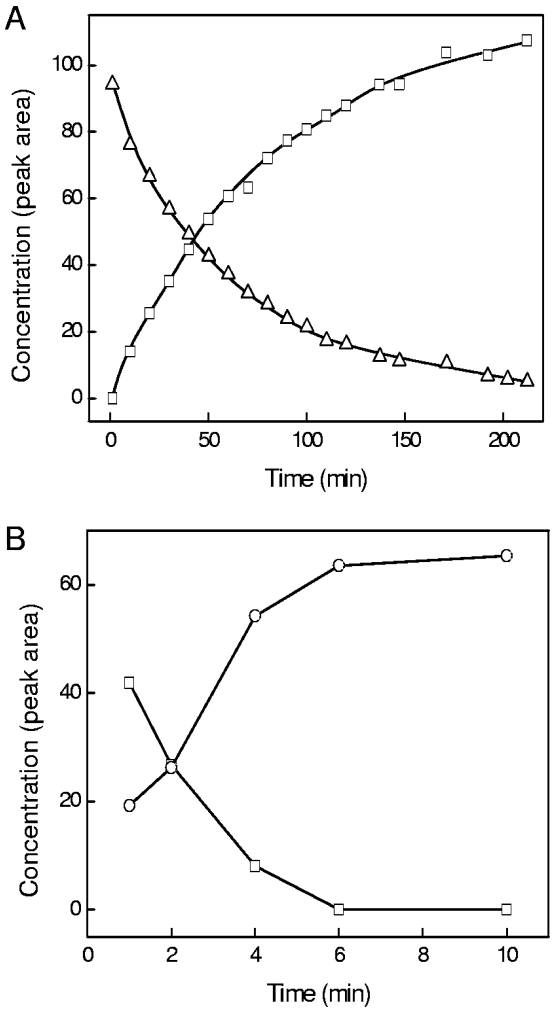

FIG. 3. Transformations of sulfolactate (△) to sulfopyruvate (□) during the reaction of sulfolactate dehydrogenase (SlcD) (A) and of sulfopyruvate (□) to sulfoacetaldehyde (○) during the reaction of sulfopyruvate decarboxylase (ComDE) (B) from *R. nubinhibens* ISM.

The second product from Xsc and also a product from CuyA is sulfite (Fig. 1A). This is presumed to be oxidized periplasmically by a sulfite dehydrogenase, some of which seems to be difficult to assay (16, 28). This enzyme was detected with extracts of cells from all cultures examined (Table 1).

The pathway predicted for the degradation of taurine involves taurine:pyruvate aminotransferase (Tpa), which is encoded separately from Xsc. Activity of this enzyme in extracts of acetate-grown, (*S*)-cysteate-grown, and sulfolactate-grown cells was detectable, but the enzyme was strongly induced in taurine-grown cells (Table 1).

An experiment was done with the crude extract of sulfolactate-grown cells to which sulfopyruvate and glutamate were added. A representative intermediate of each branch of the pathway, cysteate and sulfoacetaldehyde (0.1 mM), was formed. Both branches of the bifurcated pathway were thus in operation simultaneously.

Separation, purification, and identification of enzymes in the bifurcated pathway. Proteins in the soluble extract of sulfolactate-grown cells were loaded onto an anion-exchange column. Separated fractions with activities of Xsc, CuyA, or ComDE were examined.

All fractions containing activity of Xsc contained the characteristic 63-kDa band (SDS-PAGE) known from earlier work (e.g., reference 45); other fractions did not. The protein was subject to peptide mass fingerprinting (see Fig. S1 in the supplemental material), which confirmed that it represented the gene product of the candidate *xsc* gene (ISM_10690) (Fig. 1B; Table 2).

All fractions containing activity of CuyA contained the characteristic 35-kDa band (SDS-PAGE) known from earlier work (14); other fractions did not. Active fractions were combined and subjected to a second purification step by anion-exchange at a higher pH value. An enrichment factor of about 10-fold was achieved; the prominent 35-kDa band was excised and subjected to peptide-mass fingerprinting (not shown), which confirmed that it represented the gene product of the candidate *cuyA* gene (ISM_09626) (Fig. 1B; Table 2).

The activity of ComDE, detected as substrate disappearance (described above), was confirmed to involve the concomitant release of sulfoacetaldehyde (Fig. 3B). This product was tentatively identified, after derivatization, by cochromatography (HPLC) with derivatized authentic material. The identification was confirmed by its reaction with the specific sulfoacetaldehyde reductase (EC 1.1.1.-) of *Chromohalobacter salexigens* DSM 3043 (Z. Krejčík and A. M. Cook, unpublished data) to form isethionate, which was identified by ion chromatography.

A three-step purification involving hydrophobic interaction chromatography, anion-exchange chromatography, and gel filtration chromatography yielded the two subunits of ComDE purified to homogeneity (Fig. 4). The proteins were subject to peptide mass fingerprinting (not shown), which confirmed that the subunits were indeed the products of the candidate *comDE* genes (ISM_13335 and ISM_13330, respectively) (Fig. 1B; Table 2).

The enrichment factor was about 150-fold. The native enzyme was estimated by gel filtration chromatography to be about 235 kDa (not shown), which suggests the same $\alpha_6\beta_6$ structure (calculated as 230 kDa) found in the enzyme from the archaeon *M. jannaschii* (25). The purified enzyme was stable under an air atmosphere; it could be stored at 4°C for at least a month without loss of activity.

Membrane-associated SlcD could be solublized from the membrane and applied to an anion-exchange column, and activity was eluted from the column. However, the separated fractions represented no purification, in that the pattern of protein bands was largely identical with that of the starting material. A 10-fold increase in the specific activity of SlcD after gel filtration still did not allow a reasonable candidate protein to be postulated directly (not shown), but the presence of inducible, membrane-bound sulfolactate dehydrogenase activity and annotation of a gene as (membrane-bound) 2-hydroxyacid dehydrogenase allowed a gene candidate to be nominated (see below).

Inducible transcription of relevant genes shown by RT-PCR. The biochemical identification of four gene products (ComDE, Xsc, and CuyA) in sulfonate degradation (Fig. 1) was complemented by observing inducible transcription of the genes (*comDE*, *xsc*, and *cuyA*) by RT-PCR (not shown). The three genes downstream of *comDE* were designated *slcHFG*, and the

TABLE 2. Organisms with orthologues of SlcD, and orthologues of relevant enzymes of the bifurcated sulfolactate degradative pathway[c]

Organism	Organism tag	Locus tag encoding indicated enzyme:				
		SlcD	ComE	ComD	Xsc	CuyA
Roseovarius nubinhibens ISM	ISM_	13310	**13330**	**13335**	10690	09626
Phaeobacter gallaeciensis BS107	RGBS107_	**10911**	**10916**	**10921**	03088	05569[a]
Roseobacter denitrificans OCh 114	RD1_	**3814**	**3813**	**3812**	0826	0819
Roseobacter litoralis Och 149	RLO149_	**14678**	**14683**	**14688**	15453	15243
Roseobacter sp. strain SK 209-2-6	RSK20926_	**07142**	**07147**	**07152**	10419	14094
Rhodobacterales bacterium HTCC2083	RB2083_	**2743**	**2808**	**708**	3832	3184
Rhodobacterales bacterium Y41	RBY41_	**4170**	**4207**	**4138**	3832	1128
Roseovarius sp. strain 217	ROS217_	11241	None	None	11936	09350
Roseovarius sp. strain TM1035	RTM1035_	17047	None	None	16562	19346
Ruegeria pomeroyi DSS-3	SPO	0598	None	None	3561	A0158
Jannaschia sp. strain CCS1	Jann_	**1403**	**1402**	**1401**	2846	None
Octadecabacter antarcticus 238	OA238_	**2138**	—[b]	**1847**	2599	None
Octadecabacter antarcticus 307	OA307_	**2251**	—[b]	**3307**	4001	None
Phaeobacter gallaeciensis 2.10	RG210_	**02412**	**02407**	**02402**	10327	None
Rhodobacterales bacterium HTCC2150	RB2150_	**17119**	**17114**	**17109**	15441	None
Rhodobacterales bacterium KLH11	RKHL11_	**3493**	**3454**	**3575**	2875	None
Roseobacter sp. strain CCS2	RCCS2_	**12604**	**12609**	**12614**	04724	None
Roseobacter sp. strain MED193	MED193_	**17034**	**17039**	**17044**	12208	None

[a] A paralogous hypothetical protein (19978) is also present.
[b] —, *comE* is present but not annotated.
[c] Contiguous clusters are shown in bold type. Abbreviations of enzyme names are given in the text.

gene products were postulated to be a sulfolactate transporter in the tripartite tricarboxylate transporter (TTT) family (TC 2.A.80.-.- in the transport classification system) after using the BLASTP server on the Transport Classification Database. We could observe inducible transcription of *slcH* and *slcG* and detect the transcript of *slcF* (not shown), which we interpreted as support for the presence and presumed function of SlcHFG. The next and last gene in this cluster was designated *slcD*, because the annotation (membrane-bound 2-hydroxy-acid dehydrogenase) corresponded to properties of SlcD (membrane-bound sulfolactate dehydrogenase). The *slcD* gene was transcribed inducibly (see Fig. S2 in the supplemental material).

SlcD was considered to be another key to the bifurcated pathway shown in Fig. 1, so all orthologues, which could be identified with the BLASTP algorithm on the NCBI website, were tabulated (Table 2). The 18 sequences formed a tight cluster in a dendrogram (not shown).

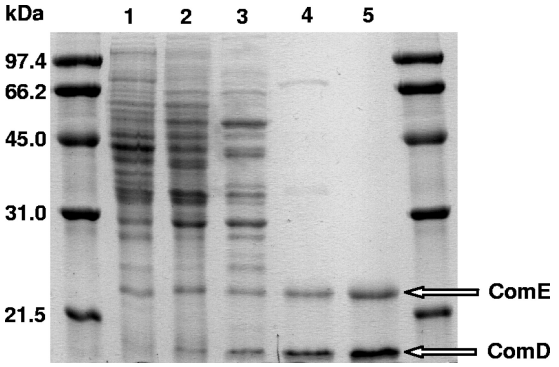

FIG. 4. Electropherogram of denatured proteins of *R. nubinhibens* ISM at different stages of purification of ComDE. The sizes of the molecular mass markers are indicated on the left. Lane 1, soluble fraction of acetate-grown cells; lane 2, soluble fraction of sulfolactate-grown cells; lane 3, active fraction after hydrophobic-interaction chromatography; lane 4, active fraction after anion-exchange chromatography; lane 5, purified enzyme after gel filtration chromatography.

The gene downstream of *xsc* is annotated as *pta*. Both *xsc* and *pta* are transcribed inducibly, corresponding to the enzyme data shown in Table 1 and supporting the role of these enzymes in sulfolactate metabolism in strain ISM (Fig. 1).

The gene downstream of *cuyA* is *cuyZ*. Each is transcribed inducibly (not shown). We conclude that CuyZ is a sulfite exporter (Fig. 1), as deduced previously (10, 14). Sulfite dehydrogenase (SorAB), which is under further study, is currently attributed to ISM_01020 and ISM_01015 (see reference 16).

DISCUSSION

The growth curve showing utilization of sulfolactate by a bacterium (Fig. 2A) is apparently the first to be published. The need to synthesize sulfolactate, which is not commercially available, presumably explains this oddity. The phase representing growth with sulfolactate shows that substrate utilization and product (sulfate) formation are concomitant with growth (Fig. 2B), which proves the stoichiometry and the mass balances in this system. Further, the derived, specific turnover rate for sulfolactate, 1.4 mkat (kg protein)$^{-1}$, is of the same order of magnitude as specific activities of relevant enzymes in the degradative process (Table 1).

Two pathways including sulfolactate degradation have been sketched in the literature. The first involves desulfonation via SuyAB (42), which is absent here (introduction; Table 1). The second involves transformation to (*S*)-cysteate (9) via oxidation and transamination. (*S*)-Cysteate is desulfonated to pyruvate by CuyA, and the export of sulfite is attributed to CuyZ (14). This second pathway is obviously functional in *R. nubinhibens* ISM (Fig. 1A, right; Table 1).

In addition, a novel (third) pathway is described above. It involves the same oxidation (to sulfopyruvate) found in the second pathway, but decarboxylation to sulfoacetaldehyde and

desulfonation by Xsc are involved (Fig. 1A, left; Table 1). The oxidation (Fig. 3A), decarboxylation (Fig. 3B), and desulfonation (Table 1) have clearly been demonstrated; the sulfopyruvate decarboxylase has been purified (Fig. 4). This degradative pathway (Fig. 1) involves some previously known components (see below), but for the first time, there is a candidate uptake system for sulfolactate, SlcHFG.

Graham et al. (23) consider that the biogenic sulfonate substituent is present to prevent a molecule from crossing a membrane, so a transport system is essential to allow biodegradation of an extracellular organosulfonate by intracellular enzymes. The candidate sulfolactate transporter, SlcHFG (Fig. 1), whose genes are inducibly transcribed (see above), is apparently a member of TC 2.A.80.-.-, whose sole defined member (TctABC) (56) has components of 504, 144, and 325 amino acid residues, with 12, 4, and 0 transmembrane helices, respectively; the predicted data for SlcHFG are 502 amino acid residues/11 transmembrane helices (SlcF), 152/4 (SlcG), and 305/0 (SlcH, a periplasmic binding protein). SlcHFG is not widespread in the organisms listed in Table 2, being present only in strain ISM and *Roseobacter* sp. strain CCS2. The *slcHFG* genes are also found in the genome sequence of *C. salexigens* DSM 3043 contiguous with the *suyAB* genes, and *C. salexigens* is found to utilize sulfolactate; we therefore consider this indirect support for the transport candidate (see the supplemental material). Available organisms lacking the *slcHFG* genes (*Roseovarius* sp. strain 217, *R. pomeroyi* DSS-3, and *Roseobacter* sp. strain MED193) do not utilize sulfolactate, and as in many organisms listed in Table 2 which contain *slcD-comED*, the latter genes are located at the end of a long gene cluster. We postulate that the sulfolactate in many organisms is generated intracellularly from a precursor, presumably 2,3-dihydroxysulfopropane (see below), which is utilized by the relevant organisms tested to date, *Ruegeria pomeroyi* DSS-3 and *Roseovarius* sp. strain 217 (K. Denger, unpublished data).

The inducible sulfolactate dehydrogenase shown in Fig. 1 is attributed by us to the gene product of ISM_13310 (*slcD*). Its annotation suggests that FMN is the electron acceptor, but the enzyme was assayed with the artificial electron acceptor, ferricyanide, because the tested flavins elicited no activity. The (*R*)-sulfolactate dehydrogenase best known in the literature is the NAD(P)-coupled EC 1.1.1.272 (ComC); ComC can also oxidize (*S*)-malate (22, 24), which is not a substrate for SlcD from strain ISM. The archaeal enzyme is involved in the biosynthesis of coenzyme M, hence the abbreviation "Com." An unsolved problem is that our chemically synthesized sulfolactate is racemic (42), whereas the compound is degraded completely (Fig. 2). We presume that SlcD is enantiomer specific, and we postulate the presence of an unknown sulfolactate racemase (or an equivalent pathway).

The following enzyme in the novel pathway, ComDE (Fig. 1A, 3B, and 4), was first characterized as an archaeal gene product which is similar in size and structure ($\alpha_6\beta_6$) but oxygen sensitive (25). The enzyme in strain ISM is stable in air, and the orthologues referred to in Table 2 all cluster in a dendrogram apart from the archaeal sequences (see Fig. S3 in the supplemental material). The function of ComDE in this pathway (Fig. 1A) is to convert a C_3 compound to a C_2 compound, which is the substrate for desulfonative Xsc (Fig. 1A). We presume that the sulfite released by Xsc is exported via CuyZ. The carbon moiety formed by Xsc, acetyl phosphate, is converted to acetyl-coenzyme A by Pta and thus made available for carbon skeletons and energy conservation via the Krebs cycle, anaplerosis via malyl-coenzyme A lyase (EC 4.1.3.24) (1), and fatty acid formation.

The pathway to desulfonation by CuyA, which operates simultaneously with the novel pathway, involves a cysteate aminotransferase (Coa) (Fig. 1A; Table 1). Literature data suggest that Coa is a known enzyme, aspartate:2-oxoglutarate aminotransferase (EC 2.6.1.1) (Aoa) (29, 57), and this hypothesis is currently being tested (J. Mayer, unpublished data).

When CuyA was discovered in several terrestrial organisms, *cuyA* had no orthologues in genome sequences (14). It is clear from the data shown in Table 2 that *cuyA* is widespread in marine bacteria. A dendrogram (see Fig. S4 in the supplemental material) indicates that some 14 CuyA orthologues are clearly separated from at least three other enzymes. We presume that CuyZ then exports the sulfite released by CuyA (Fig. 1); *cuyZ* is subject to inducible transcription under these growth conditions.

Six organisms share with *R. nubinhibens* ISM the combined genotype of *slcD-comDE*, with *xsc* and *cuyA* (Table 2). These seven organisms are presumed to degrade sulfolactate via the bifurcated pathway. Whereas *R. nubinhibens* ISM encodes SlcD and ComDE in one cluster separated by the three transporter genes (Fig. 1B), the other six organisms share contiguous *slcD-comED* genes. Xsc is encoded (with Pta) separately, as in *R. nubinhibens* ISM. CuyA is also encoded separately.

Three organisms (two *Roseovarius* spp. and *R. pomeroyi*) contain the *slcD* gene, but not the *comDE* genes, although orthologues of *xsc* and *cuyA* are present. We presume that the degradation of sulfolactate proceeds via cysteate only. *Roseovarius* sp. strain 217 and *R. pomeroyi* DSS-3 utilize (*S*)-cysteate quantitatively, but neither organism utilizes sulfolactate extensively (i.e., ~10%). None of these organisms encodes SlcHFG, so we presume that SlcD catalyzes transformation of internally generated sulfolactate. *Roseovarius* sp. strain 217 and *R. pomeroyi* DSS-3 involve Xsc in taurine metabolism (2, 21).

At least six organisms contain the contiguous *slcD-comDE* genes and the *xsc* gene, but not the *cuyA* gene (Table 2); no orthologues of *suyAB* were detected either. This is interpreted as the degradation of sulfolactate solely via Xsc. *Roseobacter* sp. strain MED193 grows with taurine, so it presumably expresses Xsc, but the organism does not utilize sulfolactate. No orthologue of SlcHFG is available, so sulfolactate is presumably generated intracellularly from a precursor (e.g., 2,3-dihydroxysulfopropane).

We feel unable to speculate on the "reason" for or the advantages of the presence of the bifurcated pathway, because many organisms grow well with a single pathway (Table 2). Each branch of the pathway is inducible individually (Table 1), so the bifurcated pathway is possibly serendipitous.

Fig. 1B shows a gene (ISM_13340) encoding a putative transcriptional regulator (SlcR; LysR type) of the six upstream genes. There are very few orthologues of this gene (see the supplemental material), possibly because most clusters involving *comDE-slcD* are larger and presumably under the control of a different regulator. This regulator is also present in *R. nubinhibens* ISM, presumably encoding enzymes to generate sulfolactate from its precursor.

We suspect that Fig. 1A represents aspects of bacterial sulfoglycolysis and expands the work of Roy et al. (44) by providing information on some fates (e.g., desulfonation) of several C_3 sulfonates. In that work, two sulfonates, sulfolactate and 2,3-dihydroxysulfopropane (44), were observed to be excreted, and we postulate that the latter is a precursor of sulfolactate in bacterial sulfoglycolysis. The term sulfoglycolysis was coined by Benson's group (3) for transformations of sulfoquinovose in plants and algae. Benson and Lee (3) describe (*S*)-cysteate as an intermediate and 2,3-dihydroxysulfopropane as an excretion product of all algae; both compounds derive from sulfoquinovose. We therefore propose that the reactions represented in Fig. 1A are bacterial models for aspects of plant and algal sulfoglycolysis. We also speculate that sulfoquinovose and derived products from phototrophs represent a significant source of carbon in the oceans, where *Pelagibacter ubique* and the *Roseobacter* clade, which contain orthologues of, e.g., *xsc*, *cuyA*, and *suyAB* (Table 2), represent >40% of the bacterial population (18). Indeed, the *Roseobacter* clade was overrepresented in association with an algal bloom (20), which was presumably (3) excreting 2,3-dihydroxysulfopropane.

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