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Sulfoacetate Is Degraded via a Novel Pathway Involving Sulfoacetyl-CoA and Sulfoacetaldehyde in *Cupriavidus necator* H16

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Bacterial degradation of sulfoacetate, a widespread natural product, proceeds via sulfoacetaldehyde and requires a considerable initial energy input. Whereas the fate of sulfoacetaldehyde in *Cupriavidus necator* (*Ralstonia eutropha*) H16 is known, the pathway from sulfoacetate to sulfoacetaldehyde is not. The genome sequence of the organism enabled us to hypothesize that the inducible pathway, which initiates *sau* (sulfoacetate utilization), involved a four-gene cluster (*sauRSTU*; H16_A2746 to H16_A2749). The *sauR* gene, divergently orientated to the other three genes, probably encodes the transcriptional regulator of the presumed *sauSTU* operon, which is subject to inducible transcription. SauU was tentatively identified as a transporter of the major facilitator superfamily, and SauT was deduced to be a sulfoacetate-CoA ligase. SauT was a labile protein, but it could be separated and shown to generate AMP and an unknown, labile CoA-derivative from sulfoacetate, CoA, and ATP. This unknown compound, analyzed by MALDI-TOF-MS, had a relative molecular mass of 889.7, which identified it as protonated sulfoacetyl-CoA (calculated 889.6). SauS was deduced to be sulfoacetaldehyde dehydrogenase (acylating). The enzyme was purified 175-fold to homogeneity and characterized. Peptide mass fingerprinting confirmed the *sauS* locus (H16_A2747). SauS converted sulfoacetyl-CoA and NADPH to sulfoacetaldehyde, CoA, and $NADP^+$, thus confirming the hypothesis.

Sulfoacetic acid, as the sulfonate ester, was first recognized as a natural product in plant alkaloids (1). Free sulfoacetate was then found to be widespread in plants and algae (2–4). The compound was also detected as an intracellular intermediate in the bacterial degradation of the plant sulfolipid (sulfoquinovosyldiacylglycerol), specifically from its polar head group, sulfoquinovose (5). This sulfolipid is nearly ubiquitous in phototrophic organisms (6) and may represent up to half of the total lipid content in some marine algae (7). The sulfur content of leaves is comprised mainly of sulfolipid and proteins; senescence of deciduous plants thus introduces significant amounts of sulfoquinovose into the soil (8). Sulfoacetate can also be the product of the bacterial assimilation of nitrogen from taurine (9, 10). The latter precursor of sulfoacetate is a major organic solute in marine creatures (11, 12) and in mammals (13), which excrete it in urine (14). Moreover, sulfoacetate is introduced into the environment as sodium lauryl sulfoacetate, a frequent ingredient of cosmetics and personal care products.

Biodegradation of sulfoacetate was first observed by Martelli and Benson (5), and evidence was presented for the hydrolytic cleavage of sulfoacetate to glycolate, but the organism was lost (15, 16). King and Quinn (17) isolated aerobic Gram-positive and Gram-negative bacteria, which degraded sulfoacetate by a different pathway, involving desulfonation via inducible sulfoacetaldehyde acetyltransferase (Xsc)[4] (EC 2.3.3.15). Various anaerobic bacteria were also found to utilize sulfoacetate via Xsc (15). The aerobic *Ralstonia* sp. strain EDS1 dissimilates a range of organosulfonates, including sulfoacetate, via Xsc, and the low molar growth yield with sulfoacetate (60% of the value for all other carbon sources) led to the conclusion that the reduction of sulfoacetate to sulfoacetaldehyde, the organic substrate of Xsc, was metabolically expensive (18). The inducible involvement of Xsc in the degradation of sulfoacetate, taurine, and isethionate was confirmed in *Cupriavidus necator* (*Ralstonia eutropha*) H16, where the common pathway also included phosphate acetyltransferase (EC 2.3.1.8), a sulfite exporter TauE (TC 9.A.29.2.1) and sulfite dehydrogenase (SorAB) (EC 1.8.2.1) (Fig. 1) (19, 20).

It was still unclear which proteins catalyzed the formation of sulfoacetaldehyde from sulfoacetate. However, although the convergence of metabolism of C_2 sulfonates at one Xsc (Fig. 1) is widespread (15), some organisms contain paralogues of *xsc*, *e.g. Desulfitobacterium hafniense* DCB-2 (Dhaf_0189 and Dhaf_4634). We showed that the presence of more than one *xsc* gene in a genome can represent complete, individual degradative pathways for different sulfonates (21). Using this idea in *D. hafniense* DCB-2, where the gene cluster upstream of one *xsc*

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[4] The abbreviation used is: Xsc, sulfoacetaldehyde acetyltransferase.

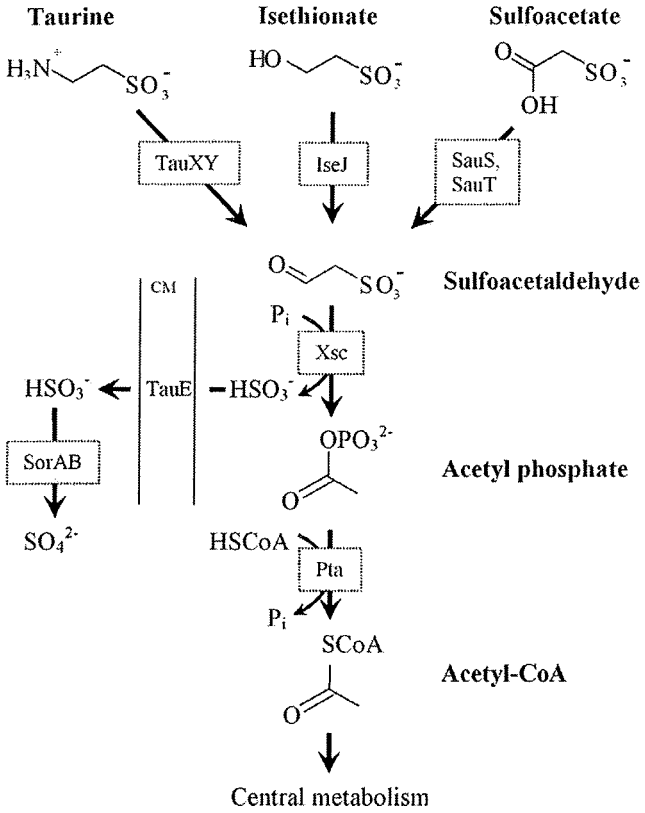

FIGURE 1. **Sulfoacetaldehyde as the point of convergence of the degradative pathways for sulfoacetate, isethionate, and taurine and the degradative pathway of sulfoacetaldehyde in *C. necator* H16.** Taurine dehydrogenase (*TauXY*) and isethionate dehydrogenase (*IseJ*) are inducible, membrane-bound, cytochrome *c*-coupled enzymes that have not been purified; TauE is a sulfite exporter, located in the cytoplasmic membrane (*CM*) (19–21, 50). *Pta*, phosphate acetyltransferase.

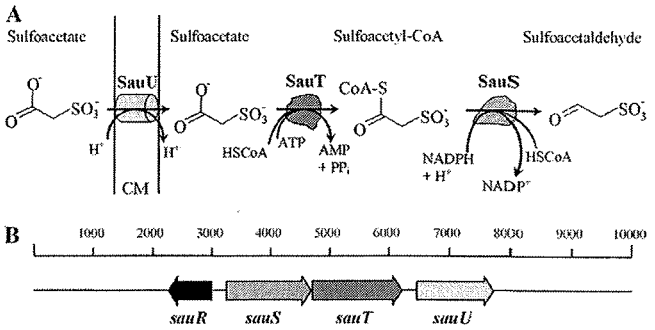

FIGURE 2. **Initial reactions in the pathway for the dissimilation of sulfoacetate in *C. necator* H16 (*A*) and the cluster of genes encoding regulated expression of pathway proteins (*B*).** The locus tags of the *sauRSTU* cluster are H16_A2746 (*sauR*) to H16_A2749 (*sauU*). *CM*, cytoplasmic membrane.

was annotated as encoding an acyl-CoA ligase and a NAD(P)-coupled aldehyde dehydrogenase (Dhaf_0190), we hypothesized that this combination of reaction types would convert sulfoacetate to sulfoacetaldehyde via putative sulfoacetyl-CoA. Orthologues of the presumptive sulfoacetaldehyde dehydrogenase (deacylating) (SauS, for sulfoacetate utilization) (Fig. 2*A*) are widespread, and one *sauS* gene is found in a four-gene cluster in *C. necator* H16. We chose to explore the *sauRSTU* cluster (Fig. 2*B*) in *C. necator* H16, with which we had relevant experience (Fig. 1). Strain H16 grows relatively fast with sulfoacetate as a sole source of carbon and energy for growth, and an established protocol to generate in-frame deletions in this organism is available (22, 23). Our hypothesis comprised an IclR-type transcriptional regulator, SauR (H16_A2746), a sulfoacetate-CoA ligase, SauT (H16_A2748), a sulfoacetaldehyde dehydrogenase (acylating), SauS (H16_A2747), and a sulfoacetate transporter belonging to the major facilitator superfamily, SauU (H16_2749) (Fig. 2*B*).

EXPERIMENTAL PROCEDURES

Materials—Sulfoacetate (99% purity) was purchased from Acros Organics (Geel, Belgium). Other commercial chemicals (~99% purity) were from Fluka (Seelze, Germany), Merck or Sigma-Aldrich. Sulfoacetyl-CoA is not available commercially, and we failed to synthesize it chemically using protocols to synthesize 3-hydroxybutyryl-CoA (24, 25). Oligonucleotides were synthesized by Microsynth (Balgach, Switzerland). Materials for (RT-)PCR and cloning were purchased from Fermentas GmbH (St. Leon-Rot, Germany) and used as provided by the supplier. Phusion DNA Polymerase from NEB (Ipswich, UK) was used during the construction of deletion mutants. Chromosomal DNA was isolated as described elsewhere (26). Total RNA was isolated using the E.Z.N.A. bacterial kit (Omega Bio-Tek, Doraville, GA). PCR products were purified using QIAquick® spin kit (Qiagen).

Bacteria, Growth Media, and Growth Conditions—*C. necator* H16 (DSM 428) (27) was isolated in Göttingen, Germany. The organism was grown at 30 °C in freshwater, mineral salt medium (28). Sulfoacetate (10–20 mM) was used routinely as the sole added source of carbon and energy for growth. For enzyme assays with the mutants, which were unable to grow with sulfoacetate, 10 mM acetate served as a growth substrate in the presence of 10 mM sulfoacetate to induce the sulfoacetate degradative enzymes.

Precultures and cultures (5 ml) for the determination of the substrate range were grown in 50-ml screw-cap tubes in a roller. Growth experiments were done on the 50-ml scale in 300-ml Erlenmeyer flasks on a shaker; samples were taken at intervals to measure optical density at 580 nm, to assay protein, and to determine the concentrations of substrate and products.

Strains of *Escherichia coli* used for site-directed mutagenesis (supplemental Table S1) were grown in LB medium at 37 °C. Appropriate antibiotics were added to medium in the following concentrations: 50 μg ml^{-1} ampicillin, 20 μg ml^{-1} tetracycline, and 5 μg ml^{-1} trimethoprim.

Preparation of Cell-free Extracts—Cell-free extracts were obtained from strain H16 grown on the 1-liter scale in 5-liter Erlenmeyer flasks. The cells were harvested in the midexponential growth phase by centrifugation (30,000 × *g*, 15 min, 4 °C), washed in 50 mM potassium phosphate buffer, pH 7.2 (containing 5 mM $MgCl_2$), and resuspended in a small volume (2–5 ml) of the same buffer. The cells were disrupted by four passages through a chilled French press set at 138 MPa, and whole cells and cell debris were removed by centrifugation (20,000 × *g*, 3 min, 4 °C). The membrane and soluble fractions were obtained by ultracentrifugation (200,000 × *g*, 30 min, 4 °C). DNA was removed by DNase, which was added prior to disruption.

Enzyme Assays—SauT was assayed discontinuously by HPLC as the formation of sulfoacetyl-CoA. The reaction mixture contained (in a final volume of 1 ml): 50 μmol of Tris/HCl buffer, pH 8.0 or 9.0 (containing 5 mM $MgCl_2$), 1 μmol of ATP, 2 μmol of sulfoacetate, 0.5 μmol of CoA, and 0.1–1 mg of protein. SauT activity was estimated as a decrease in concentration of CoA, because no reference material of sulfoacetyl-CoA was available to quantify the novel compound. Sulfoacetaldehyde dehydrogenase (acylating) (SauS) was assayed spectrophotometrically as the sulfoacetaldehyde-dependent reduction of $NADP^+$ at 365 nm, which is the reverse reaction. The reaction mixture contained (in a final volume of 1 ml): 50 μmol of Tris/HCl buffer, pH 9.0 (containing 5 mM $MgCl_2$), 1 μmol of $NADP^+$, 3 μmol of sulfoacetaldehyde, 0.5 μmol of CoA, and 1–100 μg of protein. The reaction was linear for at least 1 min.

Purification of SauS and Identification of the Corresponding Gene—The first step to purify SauS, anion exchange chromatography (MonoQ, HR 10/10; Pharmacia), was performed with the soluble protein fraction at a flow rate of 1 ml $min^{\times 1}$. An increasing gradient of sodium sulfate in 50 mM Tris/sulfate buffer, pH 8.7, was applied, and SauS was eluted at 85 mM sodium sulfate. Active fractions were combined, rebuffered on PD10 columns with 50 mM potassium phosphate buffer, pH 6.5, and loaded on to a cation exchange column (MonoS, HR 5/5; Pharmacia). An increasing gradient of sodium sulfate was applied, and SauS was eluted at 95 mM sodium sulfate. N-terminal amino acid sequencing and peptide mass fingerprinting were done by Toplab (Martinsried, Germany) on bands excised from SDS-PAGE gels.

Enzyme Separation of SauT—The first step to separate SauT, anion exchange chromatography with a MonoQ HR 10/10 column, was done with soluble fraction in 50 mM potassium phosphate buffer, pH 6.5. An increasing gradient of sodium sulfate was applied, and SauT eluted at 120 mM sodium sulfate. Fractions were desalted, concentrated, and subjected to hydroxyapatite column chromatography in 10 mM potassium phosphate buffer, pH 6.7. An increasing gradient of potassium phosphate was applied, and SauT eluted at ~100 mM potassium phosphate.

Analytical Methods—Sulfoacetate was determined by ion chromatography (9). Sulfate was quantified turbidimetrically as an insoluble suspension of $BaSO_4$ (29). Sulfite was quantified as the fuchsin adduct (30). Growth was followed turbidimetrically at 580 nm or assayed as Lowry-type protein in whole cells (31). Protein content of crude cell extracts was determined by protein dye binding (32). Denatured proteins were analyzed on 13% SDS-PAGE gels and stained with Coomassie Brilliant Blue R250 (33). The native molecular weight of separated proteins was calculated after gel filtration by interpolation in a standard curve. The values of K_m^{app} were derived by hyperbolic curve fitting.

Identification of Sulfoacetyl-CoA—Sulfoacetyl-CoA was visualized by reversed phase HPLC and a diode array detector. The stationary phase was Nucleosil 5-C18 (125 × 3 mm). The mobile phase (0.5 ml min^{-1}) was a 100 mM potassium phosphate solution, pH 5, with a gradient from 0 to 30% methanol. The identity of sulfoacetyl-CoA was confirmed by MALDI-TOF-MS. Samples of reaction mixtures containing putative sulfoacetyl-CoA generated in an assay of SauS were mixed with a matrix of saturated α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile and 1% trifluoroacetic acid. The dried droplet method was used with 0.8-μl samples on a MALDI steel target, and samples were analyzed in the negative ion mode in an Applied Biosystems 4800 MALDI-TOF/TOF mass spectrometer (34).

Cloning and Sequencing—Standard DNA techniques (35, 36) were used to isolate bacterial plasmid DNA, to transform plasmid DNA into *E. coli*, and for general DNA handling. Low throughput plasmid DNA was isolated using the QIAprep® miniprep kit, and high throughput isolation of plasmid DNA was done using a boiling method (37). Colony PCR was done as described elsewhere (38). Sequencing was done at GATC (Konstanz, Germany) or Microsynth, and the sequences were analyzed using the Lasergene package from DNASTAR (Madison, WI).

RT-PCR—The primers listed in supplemental Table S2 were used for RT-PCR. PCR was done as described elsewhere (39). The absence of DNA after isolation of RNA was tested by PCR using primers for *xsc*. Positive controls for the success of RNA isolation were done after reverse transcription using the 16 S rRNA-specific primers 16S-27F and 16S-533R (supplemental Table S2) (40). The PCR products were visualized after separation on 1.5% agarose gels.

Construction of Deletion Mutants in Individual sau Genes—Mutants of *C. necator* H16 containing in-frame deletions in one of the *sauSTU* genes were constructed by gene replacement mutagenesis as detailed in the supplemental data. Thereby, a wild type functional gene was replaced by an engineered defective short gene (see below).

Sequence Analyses and Accession Numbers—The sequence of the genome of *C. necator* H16 (accession numbers NC_008313 and NC_008314) was obtained from the National Center for Biotechnology Information, whose BLAST (41) server was also used. Sequence data were analyzed using different subroutines of the LASERGENE software package (DNASTAR), with SignalP (42), available from the Center for Biological Sequence Analysis, with PROSITE, on the ExPASy Proteomics Server, and with the BLAST server of the Transport Classification Database.

RESULTS

Growth Kinetics—C. necator H16 grew exponentially (μ = 0.14 h^{-1}) with 10–20 mM sulfoacetate as the sole source of carbon and energy (Fig. 3*A*). Growth was concomitant with substrate utilization and with the stoichiometric recovery of the sulfonate moiety as sulfate (Fig. 3*B*). The molar growth yield was 5.1 g of protein (mol C)$^{-1}$, significantly lower than the 6.0–6.6 g of protein (mol C)$^{-1}$ with taurine, isethionate, or acetate; the latter is a normal value, which indicates mass balance for carbon (43). The specific utilization rate of sulfoacetate was calculated to be 3.8 millikatals (kg of protein)$^{-1}$.

Enzyme Activities and Transcriptional Analyses in C. necator H16—The first scalar enzyme in the postulated pathway (Fig. 2*A*) was SauT. Extracts of acetate- (Table 1), taurine-, or isethionate-grown cells (not shown) showed no activity of this enzyme. Extracts of sulfoacetate-grown cells catalyzed the

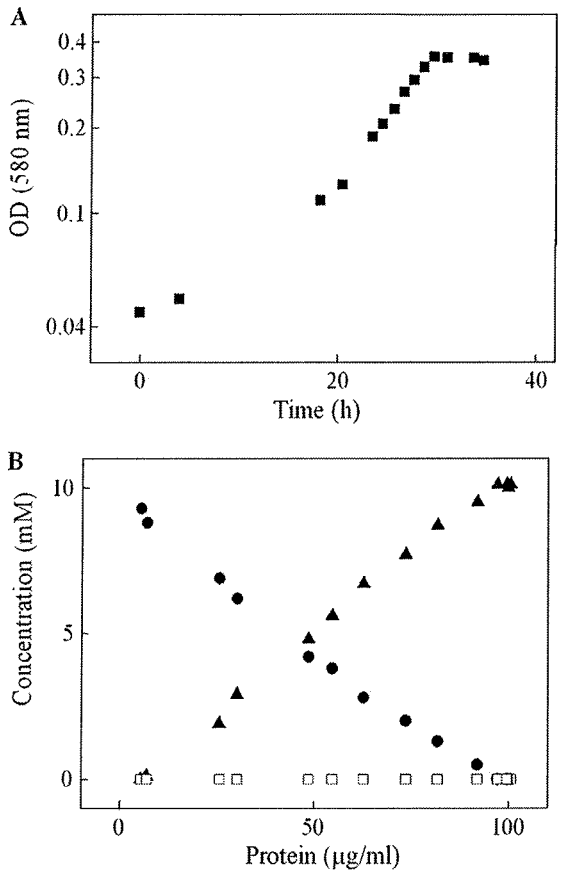

FIGURE 3. **Semi-log plot (*A*) of growth of *C. necator* H16 in 10 mM sulfoacetate-salts medium and the concentrations of substrate and products (*B*) plotted as function of protein concentration.** *Filled circles*, sulfoacetate; *filled triangles*, sulfate; *open squares*, sulfite.

TABLE 1

Activities of enzymes and transcription of genes under different growth conditions of *C. necator* H16

	Specific activity[a] or transcription	
	Cells grown with sulfoacetate	Cells grown with acetate
Transcription of *sauU* (sulfoacetate uptake)	+[b]	bdl[c]
Sulfoacetate-CoA ligase (SauT)	0.2	bdl
Transcription of *sauT*	+	bdl
Sulfoacetaldehyde dehydrogenase (SauS)	10.4	bdl
Transcription of *sauS*	+	bdl

[a] Specific enzyme activity is given in the SI unit millikatal (kg of protein)$^{-1}$.
[b] RNA transcript is scored as: −, absent; +, strong. Typical data are shown in supplemental Fig. S1.
[c] bdl, below detection limit.

CoA- and ATP-dependent conversion of sulfoacetate to sulfoacetyl-CoA (see below) and AMP (including nonspecific activity). This was interpreted as inducible activity of SauT, which was shown by ultracentrifugation of crude extract to be in the soluble fraction. The specific activity of the enzyme was low (Table 1) and difficult to quantify, because we could not stop the reaction without destroying the sulfoacetyl-CoA formed during the reaction.

The second scalar reaction was SauS (Fig. 2*A*). Extracts of acetate- (Table 1), taurine-, or isethionate-grown cells (not shown) showed no activity of this enzyme. Extracts of sulfoacetate-grown cells catalyzed the $NADP^+$- and CoA-dependent

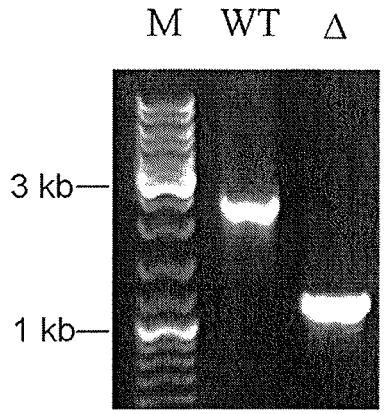

FIGURE 4. **Typical confirmation by PCR that a deletion mutant had been generated.** Amplicons of chromosomal DNA from strains H16 (*WT*) and H16Δ*sauU* (Δ) were obtained using primers h16_sauUproof_ f2 and h16_sauUproof_ r1 (supplemental Table S3). *M*, marker (GeneRuler DNA ladder mix).

conversion of sulfoacetaldehyde to sulfoacetyl-CoA (see below). This was interpreted as inducible activity of SauS, which was found to be soluble. The enzyme was highly active (Table 1), which allowed enough sulfoacetyl-CoA to be collected from the HPLC to confirm that SauS was also active in the forward reaction. SauS was identified as the gene product encoded at H16_A2747 (see below). Each of the candidate genes (Fig. 2*B*) to encode SauU (H16_A2749), SauT (H16_A2748), or SauS (H16_A2747) was found to be transcribed inducibly (Table 1), which corresponded to the inducible nature of the degradative pathway (Table 1).

Growth of Deletion Mutants—We were able to generate three deletion mutants, each with an in-frame mutation in *sauU* (H16Δ*sauU*), *sauT* (H16Δ*sauT*), or *sauS* (H16Δ*sauS*), respectively (Fig. 4). None of these mutants grew with sulfoacetate, but each grew with acetate, taurine, isethionate, or sulfoacetaldehyde, as the wild type did. The mutations were specific for sulfoacetate metabolism (Fig. 1).

Mutant H16Δ*sauU* synthesized neither SauS nor SauT (Table 2), so presumably no sulfoacetate entered the cell to enable induction to occur. In contrast, mutant H16Δ*sauT* expressed SauS (Table 2) and mutant H16Δ*sauS* expressed SauT (Table 2), so regulation of the gene cluster, presumably by SauR (Fig. 2*B*), was unaffected. No mutation in *sauR* was obtained. There appears to be irrefutable evidence for the functions of SauSTU.

Identification of Sulfoacetyl-CoA as the Intermediate in Sulfoacetate Degradation—Putative sulfoacetyl-CoA was detected by HPLC as a novel peak generated during the activation of sulfoacetate by SauT and during the oxidation of sulfoacetaldehyde by SauS. The UV spectrum of the unknown involved maxima at 212 and 257 nm and a minimum at 225 nm, similar to HSCoA but with a slightly shorter (0.7 min) retention time. The compound had a half-life of ~2 h under these conditions (pH 9.0), with shorter half-lives at higher and lower pH values. Samples taken during the reaction of purified SauS with sulfoacetaldehyde, $NADP^+$, and HSCoA were analyzed by MALDI-TOF-MS in the negative ion mode. The formation of a compound (m/z = 888.7 = $[M - 1]^-$) was detected. The value

TABLE 2

Phenotypes and enzyme activities of different mutants of *C. necator* H16

	H16Δ*sauS*	H16Δ*sauT*	H16Δ*sauU*
Growth with sulfoacetate			
Activity of sulfoacetaldehyde dehydrogenase (SauS)	bdl[a]	22.3[b]	bdl
Activity of sulfoacetate-CoA ligase (SauT)	0.03	bdl	bdl

[a] bdl, below detection limit.
[b] Specific enzyme activity is given in the SI unit millikatal (kg of protein)$^{-1}$.

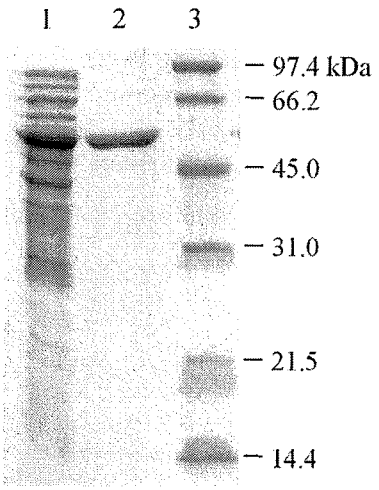

FIGURE 5. **Electropherogram of SauS at different stages of purification.** *Lane 1*, SauS after anion exchange chromatography; *lane 2*, SauS after cation exchange chromatography; *lane 3*, molecular mass markers.

of *M* (889.7) corresponds to that calculated (889.6) for protonated sulfoacetyl-CoA. This was taken as confirmation of the identity of sulfoacetyl-CoA.

Separation of SauT—Two separative steps with SauT were possible before activity was lost. This preparation showed sulfoacetate-dependent formation of sulfoacetyl-CoA and of AMP, but SauT was insufficiently concentrated to be visible on SDS-PAGE gels.

Purification and Characterization of SauS—SauS was purified 175-fold to apparent homogeneity (Fig. 5) in two steps (Table 3). The sequence of six N-terminal amino acids was determined to be SVQILH. This corresponded to the derived sequence of only one ORF (H16_A2747) in the genome of *C. necator* H16, which confirmed our hypothetical locus for *sauS* (Fig. 2*B*). Peptide mass fingerprinting of SauS confirmed this conclusion. The derived molecular mass of mature SauS, 51.5 kDa, corresponds to the value (~52 kDa) for the denatured protein (Fig. 5) interpolated into the standard curve. Separation of native SauS on a calibrated gel filtration column allowed a molecular mass of 115 kDa to be calculated. Given the errors in the method (44), we tentatively postulate that native SauS is a dimer.

SauS was specific for $NADP^+$ as a cofactor that could not be replaced by NAD^+. The K_m^{app} values for $NADP^+$, CoA, and sulfoacetaldehyde were 64, 102, and 330 μM, respectively. The enzyme was specific for sulfoacetaldehyde; none of the 10 tested compounds was a substrate: acetaldehyde, phosphonoacetaldehyde, glycolaldehyde, formaldehyde, propionaldehyde,

TABLE 3

Purification of sulfoacetaldehyde dehydrogenase (SauS) from *C. necator* H16

Purification step	Specific activity	Volume	Total protein	Recovery	Purification
	millikatals kg^{-1}	*ml*	*mg*	*%*	*fold*
Crude cell extract	7	2.1	53	100	1
Soluble fraction	9	2.0	37	94	1
Anion exchanger (pool)	35	3.5	4	37	5
Cation exchanger	1191	0.9	0.002	1	175

succinic semialdehyde, glyoxylate, betaine aldehyde, glyceraldehyde, and 2-oxobutanoic acid. None of these compounds inhibited the enzyme reaction. The optimal activity of the enzyme was obtained in 50 mM Tris/HCl buffer, pH 9.0, containing 5 mM magnesium chloride. The enzyme could be stored at 4 or −18 °C but lost 30% of its activity after 1 week.

DISCUSSION

The hypothetical pathway contains three steps, one vectorial and two scalar, and a novel metabolic intermediate, sulfoacetyl-CoA (Fig. 2*A*). Evidence for sulfoacetyl-CoA was obtained by HPLC and confirmed by MALDI-TOF-MS. Our earlier hypothesis, a phosphorylated intermediate analogous to steps in the biosynthesis of, for example, lysine or proline (18), is thus invalid. Sulfoacetyl-CoA must be almost as ubiquitous as sulfoacetate, but it will be almost undetectable in the environment, partly because it is intracellular and partly because it is so labile. We suggested that sulfoacetate is a ubiquitous natural product (see the Introduction), although there are no direct determinations of this. The biodiversity of sulfoacetate degradation[5] may be considered to support this hypothesis, because organisms have evolved and sustained so many different variants of the degradative pathway. The reactions we have established (Fig. 2) seem to be novel in degradative pathways.

The weakest portion of our hypothesis is the identification of the transcriptional regulator, SauR, attributed to locus tag H16_A2746. The gene cluster, *sauSTU*, is inducibly transcribed, and the enzymes are expressed inducibly (Table 1), so a regulator is involved. The regulator encoded adjacent to *sauSTU* was suggested as the simplest hypothesis, given the conserved *sauRSTU* cluster in all relevant betaproteobacteria.[5]

The first step in the pathway is transport, which involves SauU in *C. necator* H16 (Fig. 2). The cytoplasmic membrane is apparently impermeable for sulfoacetate unless a transporter is present, because deletion mutant H16Δ*sauU* is obviously unable to induce expression of the *sau* cluster (Table 2). This emphasizes the impermeability of membranes to sulfonates pointed out elsewhere (45, 46) and supports the idea that nature uses the sulfonate substituent to prevent a molecule crossing a membrane. The sequence of SauU indicates that it belongs to the major facilitator superfamily (TC 2.A.1.-.-) of transporters. The closest orthologues (TC 2.A.1.14.-) have ~30% sequence identity and share the predicted structure of 12 transmembrane helices.

Sulfoacetate-CoA ligase, SauT, was separated sufficiently to allow us to confirm a systematic name, sulfoacetate:CoA ligase

[5] S. Weinitschke, M. Buhmann, T. H. M. Smits, and A. M. Cook, manuscript in preparation.

(AMP-forming) (EC 6.2.1.-). This enzyme represents one major energy drain in the pathway (with SauS as another) compared with, for example, taurine or isethionate (Fig. 2*A*), given that each named growth substrate yields only one acetyl-CoA for energy conservation and anabolism (Fig. 1). This degradative pathway (Fig. 2*A*) makes it easy to understand how 20–40% (this paper and Ref. 18) reductions in molar growth yield occur.

Sulfoacetaldehyde dehydrogenase (acylating) (SauS), apparently the marker enzyme for the pathway,[5] has been purified in this study. It shows the highest similarity to aldehyde dehydrogenases (acylating) (EC 1.2.1.10), a reaction it does not catalyze. (Acet)aldehyde dehydrogenases (EC 1.2.1.-) (47) are thought to be NAD^+-dependent enzymes (also reacting with $NADP^+$ but at a lower rate), existing as dimers (48), tetramers, or polymers. Purified sulfoacetaldehyde dehydrogenase (acylating) from *C. necator* H16 is presumed to form a dimer in solution but acts solely with $NADP^+$ as a cofactor, and sulfoacetaldehyde and CoA act as substrates. SauS must be distinguished both from sulfoacetaldehyde dehydrogenase, SafD (EC 1.2.1.73), which is specific for NAD^+ and generates sulfoacetate from sulfoacetaldehyde (10), and from sulfoacetaldehyde reductase, IsfD (EC 1.1.1.-), which generates isethionate from sulfoacetaldehyde and $NADP^+$ (49). We propose that SauS, nonidentical with any reported enzyme, should have the accepted name "sulfoacetaldehyde dehydrogenase (acylating)" and the systematic name "2-sulfoacetaldehyde:$NADP^+$ oxidoreductase (CoA-acylating)" (EC 1.2.1.-).

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REFERENCES

1. Folkers, K., Koniuszy, F., and Shavel, J. (1944) *J. Am. Chem. Soc.* **66,** 1083–1087
2. Gupta, S. D., and Sastry, P. S. (1988) *Arch. Biochem. Biophys.* **260,** 125–133
3. Lee, R. F., and Benson, A. A. (1972) *Biochim. Biophys. Acta.* **261,** 35–37
4. Shibuya, I., Yagi, T., and Benson, A. A. (1963) in *Studies on Microalgae and Photosynthetic Bacteria,* pp. 627–636, The University of Tokyo Press, Tokyo
5. Martelli, H. L., and Benson, A. A. (1964) *Biochim. Biophys. Acta.* **93,** 169–171
6. Harwood, J. L. (1980) in *The Biochemistry of Plants* (Stumpf, P. K., ed) pp. 301–320, Academic Press, New York
7. Dembitsky, V. M., Rozentsvet, O. A., and Pechenkina, E. E. (1990) *Phytochemistry* **29,** 3417–3421
8. Harwood, J. L., and Nicholls, R. G. (1979) *Biochem. Soc. Trans.* **7,** 440–447
9. Denger, K., Weinitschke, S., Hollemeyer, K., and Cook, A. M. (2004) *Arch. Microbiol.* **182,** 254–258
10. Krejcík, Z., Denger, K., Weinitschke, S., Hollemeyer, K., Paces, V., Cook, A. M., and Smits, T. H. (2008) *Arch. Microbiol.* **190,** 159–168
11. Allen, J. A., and Garrett, M. R. (1971) *Adv. Mar. Biol.* **9,** 205–253
12. Yin, M., Palmer, H. R., Fyfe-Johnson, A. L., Bedford, J. J., Smith, R. A., and Yancey, P. H. (2000) *Physiol Biochem. Zool.* **73,** 629–637
13. Huxtable, R. J. (1992) *Physiol. Rev.* **72,** 101–163
14. Stipanuk, M. H. (2004) *Annu. Rev. Nutr.* **24,** 539–577
15. Cook, A. M., and Denger, K. (2002) *Arch. Microbiol.* **179,** 1–6
16. Martelli, H. L., and Souza, S. M. (1970) *Biochim. Biophys. Acta.* **208,** 110–115
17. King, J. E., and Quinn, J. P. (1997) *Microbiology* **143,** 3907–3912
18. Denger, K., and Cook, A. M. (2001) *Arch. Microbiol.* **176,** 89–95
19. Denger, K., Weinitschke, S., Smits, T. H., Schleheck, D., and Cook, A. M. (2008) *Microbiology* **154,** 256–263
20. Weinitschke, S., Denger, K., Cook, A. M., and Smits, T. H. (2007) *Microbiology* **153,** 3055–3060
21. Weinitschke, S., Sharma, P. I., Stingl, U., Cook, A. M., and Smits, T. H. (2010) *Appl. Environ. Microbiol.* **76,** 618–621
22. Lenz, O., Schwartz, E., Dernedde, J., Eitinger, M., and Friedrich, B. (1994) *J. Bacteriol.* **176,** 4385–4393
23. Jeffke, T., Gropp, N. H., Kaiser, C., Grzeszik, C., Kusian, B., and Bowien, B. (1999) *J. Bacteriol.* **181,** 4374–4380
24. Chohan, S. N., and Copeland, L. (1998) *Appl. Environ. Microbiol.* **64,** 2859–2863
25. Stadtman, T. C., and Elliott, P. (1957) *J. Biol. Chem.* **228,** 983–997
26. Desomer, J., Crespi, M., and Van Montagu, M. (1991) *Mol. Microbiol.* **5,** 2115–2124
27. Pohlmann, A., Fricke, W. F., Reinecke, F., Kusian, B., Liesegang, H., Cramm, R., Eitinger, T., Ewering, C., Pötter, M., Schwartz, E., Strittmatter, A., Voss, I., Gottschalk, G., Steinbüchel, A., Friedrich, B., and Bowien, B. (2006) *Nat. Biotechnol.* **24,** 1257–1262
28. Thurnheer, T., Köhler, T., Cook, A. M., and Leisinger, T. (1986) *J. Gen. Microbiol.* **132,** 1215–1220
29. Sörbo, B. (1987) *Methods Enzymol.* **143,** 3–6
30. Ruff, J., Denger, K., and Cook, A. M. (2003) *Biochem. J.* **369,** 275–285
31. Kennedy, S. I., and Fewson, C. A. (1968) *Biochem. J.* **107,** 497–506
32. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254
33. Laemmli, U. K. (1970) *Nature* **227,** 680–685
34. Hollemeyer, K., Velagapudi, V. R., Wittmann, C., and Heinzle, E. (2007) *Rapid Commun. Mass. Spectrom.* **21,** 336–342
35. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) *Current Protocols in Molecular Biology,* pp. 2.0.1–2.1.10, John Wiley & Sons, New York
36. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* 2nd Ed., pp. 1.21–1.32, 1.74–1.84, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
37. Holmes, D. S., and Quigley, M. (1981) *Anal. Biochem.* **114,** 193–197
38. Coenye, T., Goris, J., Spilker, T., Vandamme, P., and LiPuma, J. J. (2002) *J. Clin. Microbiol.* **40,** 2062–2069
39. Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J. (1990) *PCR Protocols: A Guide to Methods and Applications,* pp. 3–12, Academic Press, Inc., San Diego
40. Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. (1991) *J. Bacteriol.* **173,** 697–703
41. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) *Nucleic Acids Res.* **25,** 3389–3402
42. Bendtsen, J. D., Nielsen, H., von Heijne, G., and Brunak, S. (2004) *J. Mol. Biol.* **340,** 783–795
43. Cook, A. M. (1987) *FEMS Microbiol. Rev.* **46,** 93–116
44. le Maire, M., Ghasi, A., and Moller, J. V. (1996) *ACS Symp. Ser.* **635,** 36–51
45. Graham, D. E., Xu, H., and White, R. H. (2002) *J. Biol. Chem.* **277,** 13421–13429
46. Mampel, J., Maier, E., Tralau, T., Ruff, J., Benz, R., and Cook, A. M. (2004) *Biochem. J.* **383,** 91–99
47. Powlowski, J., Sahlman, L., and Shingler, V. (1993) *J. Bacteriol.* **175,** 377–385
48. Söhling, B., and Gottschalk, G. (1993) *Eur. J. Biochem.* **212,** 121–127
49. Krejcík, Z., Hollemeyer, K., Smits, T. H., and Cook, A. M. (2010) *Microbiology* **156,** 1547–1555
50. Brüggemann, C., Denger, K., Cook, A. M., and Ruff, J. (2004) *Microbiology* **150,** 805–816