2,3-Dihydroxypropane-1-sulfonate degraded by *Cupriavidus pinatubonensis* JMP134: purification of dihydroxypropanesulfonate 3-dehydrogenase

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2,3-Dihydroxypropane-1-sulfonate (DHPS) is a widespread intermediate in plant and algal transformations of sulfoquinovose (SQ) from the plant sulfolipid sulfoquinovosyl diacylglycerol. Further, DHPS is recovered quantitatively during bacterial degradation of SQ by *Klebsiella* sp. strain ABR11. DHPS is also a putative precursor of sulfolactate in e.g. *Ruegeria pomeroyi* DSS-3. A bioinformatic approach indicated that some 28 organisms with sequenced genomes might degrade DHPS inducibly via sulfolactate, with three different desulfonative enzymes involved in its degradation in different organisms. The hypothesis for *Cupriavidus pinatubonensis* JMP134 (formerly *Ralstonia eutropha*) involved a seven-gene cluster (Reut_C6093–C6087) comprising a LacI-type transcriptional regulator, HpsR, a major facilitator superfamily uptake system, HpsU, three NAD(P)⁺-coupled DHPS dehydrogenases, HpsNOP, and (*R*)-sulfolactate sulfo-lyase (SuyAB) [EC 4.4.1.24]. HpsOP effected a DHPS-racemase activity, and HpsN oxidized (*R*)-DHPS to (*R*)-sulfolactate. The hypothesis for *Roseovarius nubinhibens* ISM was similar, but involved a tripartite ATP-independent transport system for DHPS, HpsKLM, and two different desulfonative enzymes, (*S*)-cysteate sulfo-lyase [EC 4.4.1.25] and sulfoacetaldehyde acetyltransferase (Xsc) [EC 2.3.3.15]. Representative organisms were found to grow with DHPS and release sulfate. *C. pinatubonensis* JMP134 was found to express at least one NAD(P)⁺-coupled DHPS dehydrogenase inducibly, and three different peaks of activity were separated by anion-exchange chromatography. Protein bands (SDS-PAGE) were subjected to peptide-mass fingerprinting, which identified the corresponding genes (*hpsNOP*). Purified HpsN converted DHPS to sulfolactate. Reverse-transcription PCR confirmed that *hpsNOP* were transcribed inducibly in strain JMP134, and that *hpsKLM* and *hpsNOP* were transcribed in strain ISM. DHPS degradation is widespread and diverse, implying that DHPS is common in marine and terrestrial environments.

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INTRODUCTION

2,3-Dihydroxy-1-propanesulfonate (DHPS; sulfopropane-2,3-diol) advanced from a hypothetical intermediate (Benson

& Shibuya, 1961), to a tentatively identified intermediate (Shibuya *et al.*, 1963), to an identified intermediate (Busby, 1966), to a widespread plant intermediate (Lee & Benson, 1972) and an excretion product of all diatoms (Benson & Lee, 1972). The source of this intermediate is sulfoquinovose (SQ; 6-deoxy-6-sulfoglucose), the headgroup of the plant sulfolipid sulfoquinovosyl diacylglycerol (e.g. Benson, 1963), in a process termed sulfoglycolysis (Benson & Shibuya, 1961). DHPS is also an intermediate in the bacterial degradation of SQ, whereby *Klebsiella* sp. strain ABR11 initially excretes DHPS quantitatively (Roy *et al.*, 2003). The fate of DHPS after prolonged incubation was partly sulfolactate, partly sulfate and largely unknown, but working with the name ‘sulfoglycolysis’, a logical pathway of SQ degradation was hypothesized (Roy *et al.*, 2003).

Abbreviations: ComDE, sulfopyruvate decarboxylase [EC 4.1.1.79]; CuyA, (*S*)-cysteate sulfo-lyase [EC 4.4.1.25]; DHPS, 2,3-dihydroxypropane-1-sulfonate; HpsKLM, TRAP-T transporter for DHPS; HpsO and HpsP, DHPS 2-dehydrogenases of opposite stereochemistry; HpsN, DHPS 3-dehydrogenase (sulfolactate forming); HpsU, DHPS uptake; SlcD, sulfolactate dehydrogenase; SQ, sulfoquinovose; SuyAB, sulfolactate sulfo-lyase [EC 4.4.1.24]; Xsc, sulfoacetaldehyde acetyltransferase [EC 2.3.3.15].

Supplementary material, with details of the chemical synthesis of (*R,S*)-DHPS, bacteria with *hps* genes, and RT-PCR primers, is available with the online version of this paper.

Work on the degradation of (R,S)-3-sulfolactate has yielded four degradative pathways involving three desulfonation reactions: (R)-sulfolactate sulfo-lyase (SuyAB) [EC 4.4.1.24] (Denger & Cook, 2010; Rein *et al.*, 2005), (S)-cysteate sulfo-lyase (CuyA) [EC 4.4.1.25] (Denger *et al.*, 2006) and sulfoacetaldehyde acetyltransferase (Xsc) [EC 2.3.3.15] (Denger *et al.*, 2009; Ruff *et al.*, 2003). The pathways are: (i) oxidation to sulfo-pyruvate and transamination to allow desulfonation by CuyA (Denger *et al.*, 2009), (ii) oxidation to sulfo-pyruvate and decarboxylation to allow desulfonation by Xsc – both (i) and (ii) are branches of the bifurcated sulfolactate degradative pathway identified in *Roseovarius nubinhibens* ISM (Denger *et al.*, 2009), (iii) direct desulfonation via SuyAB in *Paracoccus pantotrophus*, whereby the racemase is unknown (Denger *et al.*, 2009; Rein *et al.*, 2005), and (iv) the function of two sulfolactate dehydrogenases (ComC and SlcC) of opposite stereochemistry to generate the substrate for SuyAB in *Chromohalobacter salexigens* (Denger & Cook, 2010).

The work on the degradation of (R,S)-3-sulfolactate in *Roseovarius nubinhibens* ISM and other organisms led us to suspect that a widespread gene cluster (Fig. 1b) encoded inducible enzymes for the uptake of DHPS and its conversion to sulfolactate (Denger *et al.*, 2009). We thus hypothesize (Fig. 1a, b) that a tripartite ATP-independent transport system (TRAP-T) [TC 2.A.56.–.] pumps DHPS into the cell, where two dehydrogenases are responsible for conversion to the correct DHPS enantiomer, whose primary alcohol is subject to oxidation to (2R)-sulfolactate via a third dehydrogenase. The sulfolactate is then desulfonated via the characterized bifurcated pathway (Denger *et al.*, 2009).

We now present evidence, including enzyme purification, to support the pathway (Fig. 1a), with different transporters and desulfonative enzymes, in *Roseovarius nubinhibens* ISM and *Cupriavidus pinatubonensis* JMP134 (formerly *Ralstonia eutropha*). Further, there are indications of biodiversity in transport systems in other organisms.

METHODS

Chemical synthesis of DHPS and the sources of other chemicals. The two-step synthesis of (R,S)-DHPS (sodium salt) $[\text{CH}_2(\text{OH})\text{-CH}(\text{OH})\text{-CH}_2\text{-SO}_3\text{Na}]$ via barium bispropane-2,3-diol-sulfonate $[\text{CH}_2(\text{OH})\text{-CH}(\text{OH})\text{-CH}_2\text{-SO}_3^-]_2\text{Ba}^{2+}$ from allylic alcohol $\text{CH}_2=\text{CH-CH}_2\text{OH}$ is based on an old procedure described in old-fashioned German (Frieze, 1938). The method, which has a 79 % yield and takes about 2 days with modern apparatus, is described in English in the supplementary material available with the online version of this paper. NMR data confirming the synthesis of high-quality DHPS are shown in Fig. 2.

Racemic sulfolactate was synthesized as described elsewhere (Roy *et al.*, 2003), as was the bisulfite addition complex of sulfoacetaldehyde (Denger *et al.*, 2001). Commercial chemicals were of the highest purity available from Sigma-Aldrich, Fluka, Roth, Merck or Biomol. Taq DNA polymerase, Moloney murine leukaemia virus reverse transcriptase and RNase-free DNase were from Fermentas. The E.Z.N.A. bacterial RNA kit (Omega Bio-Tek) was used for RNA

preparation. Oligonucleotides were synthesized by Microsynth (Balgach, Switzerland).

Organisms and growth media. *Cupriavidus pinatubonensis* JMP134 (DSM 4058; formerly *Ralstonia eutropha*) was grown in a phosphate-buffered mineral salts medium, pH 7.2 (Thurnheer *et al.*, 1986). The marine organisms *Hoeflea phototrophica* DFL-43 (DSM 17068) (Biebl *et al.*, 2006), *Roseobacter denitrificans* OCh 114 (DSM 7001) (Shiba, 1991), *Roseovarius nubinhibens* ISM (DSM 15170) and *Ruegeria pomeroyi* DSS-3 (DSM 15171) (González *et al.*, 2003) were cultured in modified *Silicibacter* basal medium (Denger *et al.*, 2006). A supplement of yeast extract was required by strains DFL-43 (0.01 %), OCh 114 and ISM (0.05 %) (Denger *et al.*, 2009). *Roseobacter* sp. strain MED193 (J. Pinhassi, University of Kalmar, Sweden) and *Roseovarius* sp. strain 217 (Schäfer *et al.*, 2005) were grown in Tris-buffered artificial seawater (Krejčík *et al.*, 2008) with vitamins (Pfennig, 1978). All strains were grown aerobically at 30 °C, with the exception of strain MED193, which was grown at 25 °C. Either 10 mM DHPS or 15 mM acetate was added to the appropriate medium as sole source of carbon and energy. Either 20 mM or 10 mM (for marine organisms) ammonium chloride was used as nitrogen source. Precultures (3 ml) were grown in 30 ml screw-cap tubes in a roller. Cultures for enzyme assays and for protein separations were grown, harvested at an optical density at 580 nm (OD_{580}) of 0.6–0.8, washed and disrupted in a French pressure cell as described previously (Mayer & Cook, 2009). Cultures for total RNA preparation were harvested in the early exponential growth phase at OD_{580} values of about 0.3 (*C. pinatubonensis* JMP134) or about 0.46–0.49 (*Roseovarius nubinhibens* ISM).

Analytical methods. Growth was followed as OD_{580} . Sulfate was quantified turbidimetrically as a suspension of BaSO_4 (Sörbo, 1987), sulfite as the fuchsin adduct (Denger *et al.*, 2001). Acetyl phosphate was determined as iron(III) acetyl hydroxamate (Racker, 1962). Sulfolactate was quantified by ion chromatography with suppression (Denger *et al.*, 2004). Matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) in the negative-ion mode was used to confirm the identity of sulfolactate formed in the reaction of HpsN with DHPS (Tholey *et al.*, 2002). Denatured proteins were separated on 13 % SDS-PAGE gels and stained with Coomassie brilliant blue R250 (Laemmli, 1970). Quantification of protein bands was done using the Gel Doc XR gel documentation system (Bio-Rad). Peptide mass fingerprint analyses were done by the proteomics facility of the University of Konstanz.

RT-PCR experiments were done as described previously (Krejčík *et al.*, 2008), with the primers listed in Supplementary Table S2. Before reverse transcription, RNA preparations were tested for residual DNA using primer pairs JMP-hpsN-f/r for *C. pinatubonensis*, and ISM-hpsN-f/r for *Roseovarius nubinhibens* ISM. Chromosomal DNA of strains JMP134 and ISM, which was used in PCR positive controls, was isolated as described elsewhere (Desomer *et al.*, 1991). PCR products were visualized on 1.5 % agarose gels.

Enzyme assays. Activity of DHPS dehydrogenases was assayed photometrically (340 nm) at 30 °C as the formation of NAD(P)H. The reaction mixture contained 10 mM DHPS, 4 mM NAD(P)⁺ and 1–200 µg protein ml^{−1} in 50 mM Tris/HCl, pH 9.0. In addition, activity of the sulfolactate-forming DHPS 3-dehydrogenase was measured discontinuously by quantification of sulfolactate (ion chromatography). Samples (20 µl) taken from the enzyme reaction were injected directly on to the ion chromatographic column, where the eluent at pH 11 stopped the reaction. The use of hydrazine (0.1–0.4 M) in order to increase the activity of the NAD⁺-coupled DHPS 2-dehydrogenase HpsP had no significant effects. Kinetic constants were determined with reaction mixtures containing various DHPS concentrations and 4 mM NAD⁺, or with various NAD⁺ concen-

Fig. 1. (a) The conversion of DHPS to (R)-sulfolactate and the three possible desulfonation reactions found in different organisms; (b) gene clusters encoding the relevant enzymes in *C. pinatubonensis* JMP134 and *Roseovarius nubinhibens* ISM, with locus tags and our annotations.

Fig. 2. Heteronuclear single-quantum coherence-NMR spectrum of DHPS (free acid) (D_6 -DMSO).

trations and 10 mM DHPS. The pH optimum of HpsN was determined in a buffer system with constant ionic strength, composed of *N*-(2-acetamido)-2-aminoethanesulfonate, Tris and ethanolamine (Ellis & Morrison, 1982). Sulfolactate sulfo-lyase (SuyAB, EC 4.4.1.24) and (*S*)-cysteate sulfo-lyase (CuyA, EC 4.4.1.25) were assayed as release of sulfite from sulfolactate (SuyAB) (Rein *et al.*, 2005) or from (*S*)-cysteate (CuyA), respectively (Denger *et al.*, 2006). Sulfoacetaldehyde acetyltransferase (Xsc, EC 2.3.3.15) was assayed as the formation of acetyl phosphate or sulfite from sulfoacetaldehyde (Ruff *et al.*, 2003).

Protein separations. The membrane/particulate fraction was removed from the crude extract by ultracentrifugation (170 000 *g* for 30 min at 4 °C). The supernatant fluid was designated the soluble fraction, which was subject to anion-exchange chromatography (Mono Q HR 10/10; Pharmacia). A three-step gradient of sodium sulfate (0–75 mM in 10 min followed by 75–150 mM in 45 min and 150–500 mM in 10 min) in 50 mM potassium phosphate buffer (pH 7.2) was applied at a flow rate of 1 ml min⁻¹. Three DHPS dehydrogenase activities were eluted. Activity of SuyAB was detected in fractions which eluted at 100 mM sodium sulfate. Active fractions of HpsN were pooled and brought to 1.7 M ammonium sulfate, the precipitate was spun off, and the supernatant was subjected to hydrophobic interaction chromatography (Phenyl Superose HR 10/10; Pharmacia). In a linear decreasing gradient of ammonium sulfate in 20 mM potassium phosphate buffer (pH 7.2), HpsN eluted after the end of the gradient at 0 mM ammonium sulfate. The rebuffered and concentrated active fraction was loaded on to a gel filtration column (Superose 12 HR 10/30; Pharmacia) in 20 mM potassium phosphate buffer (pH 7.2) with 150 mM sodium sulfate, at a flow rate of 0.4 ml min⁻¹. Standard high-molecular-mass proteins were used for calibration, and the molecular mass of native HpsN was estimated by interpolation in the standard curve. Concentration of protein solutions was done with Vivaspin concentrators (10 kDa cut-off, PES membrane; Sartorius); PD10 columns (Sephadex G-25; GE Healthcare) were used for buffer exchanges.

Bioinformatic procedures. Analysis of the genome sequence of *C. pinatubonensis* JMP134 (accession no. CP000090, chromosome 1; CP000091, chromosome 2; CP000092, megaplasmid; CP000093, plasmid 1) and the draft genome sequence (accession no.

NZ_AALY000000000) of *Roseovarius nubinhibens* ISM were done using the BLAST algorithm on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>). Subroutines from the LASERGENE program package (DNASTAR) were used for handling sequence data, which were in the public domain before 22 December 2009. Primers for (RT-)PCR were designed using the program Amplify (version 1.2).

RESULTS

DHPS as a sole source of carbon and energy for genome-sequenced bacteria

We deduced that at least 28 bacteria (Supplementary Table S1), whose genomes have been sequenced, could utilize DHPS via the reactions shown in Fig. 1, and we tested seven representative organisms for growth (Table 1). All organisms were outgrown within 3–8 days, and they released sulfate into the growth medium. The sulfate excretion represented either about 50 % (*Ruegeria pomeroyi*) or about 100 % recovery of the sulfate concentration expected from complete desulfonation of the DHPS originally present in the growth medium. Each organism was also grown with a non-sulfonated substrate (acetate). In all relevant cases (allowing for growth in clumps), the molar growth yield with DHPS [OD₅₈₀ (mol C)⁻¹] was about 75–100 % of the control with acetate, except for *Ruegeria pomeroyi*, where the molar yield with DHPS was half that of the control. The general hypothesis, that HpsNOP were responsible for the reactions in the degradative pathway of DHPS, was still valid.

Desulfonation reactions in different organisms

We predicted that *C. pinatubonensis* JMP134 would induce SuyAB for the desulfonation in the DHPS pathway (Table 1; Supplementary Table S1). Inducible SuyAB

Table 1. Growth of representative bacteria with DHPS (or acetate) as sole source of carbon and energy, evaluated as release of sulfate from the sulfonate group and as the growth yield (compared with the control), and the desulfonation reaction(s) potentially available to the organism

Organism	Sulfate formed (% of theory)	Molar growth yield [OD ₅₈₀ (mol C) ⁻¹]		Xsc (locus tag/tags)	CuyA (locus tag)	Suy(A)B (locus tag)
		DHPS	Acetate			
<i>Cupriavidus pinatubonensis</i> JMP134	100	0.029	0.027	Reut_B3773	n.o.d.	Reut_C6087
<i>Hoeflea phototrophica</i> DFL-43	86	0.018	0.024	HPDFL43_18962	n.o.d.	HPDFL43_17096
<i>Roseobacter denitrificans</i> OCh 114	100	0.019*	0.024	RD1_0977/0826	n.o.d.	n.o.d.
<i>Roseobacter</i> sp. strain MED193	90	0.012*	0.022	MED193_12208	n.o.d.	n.o.d.
<i>Roseovarius nubinhibens</i> ISM	100	0.027	0.023	ISM_10690	ISM_09626	n.o.d.
<i>Roseovarius</i> sp. strain 217	84	0.020	0.019	ROS217_11936	ROS217_09350	n.o.d.
<i>Ruegeria pomeroyi</i> DSS-3	55	0.013	0.021	SPO3561	SPOA0158	n.o.d.

n.o.d., no orthologue detected.

*Grew in clumps, whereas the acetate control grew in suspended culture.

activity was detected [0.26 mkat (kg protein)⁻¹] in crude cell extracts. Characteristic for SuyAB is a highly expressed 42 kDa protein band (SDS-PAGE) (SuyB) in induced cells (Denger & Cook, 2010; Rein *et al.*, 2005), and a peptide-mass fingerprint of this band (see below) confirmed its identity as Reut_C6087, as predicted in Table 1.

Similarly, we predicted that *Roseovarius nubinhibens* ISM would induce both Xsc and CuyA for the desulfonation of DHPS, because this was already known for sulfolactate catabolism (Denger *et al.*, 2009). Inducible activities of Xsc [1.4 mkat (kg protein)⁻¹] and CuyA [0.41 mkat (kg protein)⁻¹] were observed in crude extracts. Activities of SuyAB (strain JMP134), Xsc and CuyA (strain ISM) were not detectable in extracts of acetate-grown cells.

DHPS dehydrogenases

Three reactions in the pathway are hypothesized as NAD(P)⁺-coupled DHPS dehydrogenases (Fig. 1). We could measure an inducible NAD⁺-coupled DHPS-dehydrogenase activity of 3.2 mkat (kg protein)⁻¹ in extracts of *C. pinatubonensis*, and of 0.6 mkat (kg protein)⁻¹ in extracts of *Roseovarius nubinhibens*. The levels of activity in extracts of non-induced cells were 10 % or less [0.06 mkat (kg protein)⁻¹ in extracts of both strains] of those in induced cells. Activities with NADP⁺ as electron acceptor were detected at lower levels.

Proteins in the soluble fraction of extracts of DHPS-grown cells of *C. pinatubonensis* were separated on an anion-exchange column. Three different peaks of DHPS dehydrogenase activity were observed, which eluted at 60, 110 and 120 mM Na₂SO₄ in the salt gradient (Fig. 3). The proteins in these active fractions were subject to SDS-PAGE, and major relevant protein bands were subject to peptide-mass fingerprinting. The major protein, which eluted at 60 mM Na₂SO₄, had a molecular mass of 47 kDa,

as predicted for HpsN, and this was confirmed by the peptide-mass fingerprint (Reut_C6092); the enzyme was NAD⁺-linked. Several major protein bands eluted from the anion exchanger at 110 mM Na₂SO₄, but the one at 35 kDa was a candidate HpsP, and this was confirmed by the peptide-mass fingerprint (Reut_C6089); the enzyme was NAD⁺-linked. A major band eluted from the anion exchanger at 120 mM Na₂SO₄ with a molecular mass of 27 kDa, and was thus a candidate HpsO. This was confirmed by the peptide-mass fingerprint (Reut_C6091). The enzyme was NADP⁺-linked.

Fig. 3. Separation of DHPS dehydrogenases from *C. pinatubonensis* JMP134 on an anion-exchange column. Solid line, absorption of eluting protein at 280 nm; dashed line, Na₂SO₄ gradient (maximum=0.5 M); black bars, specific activity of NAD⁺-dependent HpsP; checkered bars, specific activity of NAD⁺-dependent HpsN; striped bars, specific activity of NADP⁺-dependent HpsO.

DHPS 3-dehydrogenase

HpsN, separated from *C. pinatubonensis* on the anion-exchanger column, was purified to near homogeneity (97 %) (Fig. 4) via hydrophobic interaction and gel filtration columns (Table 2). The purification factor of about 29 indicated that HpsN represented about 3 % of total cell protein (and 5 % of soluble protein), which can be seen clearly in the SDS-PAGE (lane 2 in Fig. 4). The gradual loss of specific activity (and total activity) as the enzyme reached purity indicated a labile enzyme, whose activity was soon lost. The retention time on the calibrated gel filtration column indicated a molecular mass of 75 kDa for the native protein. Compared with the 47 kDa monomer, the data were tentatively interpreted to mean homodimeric HpsN.

HpsN converted DHPS to sulfolactate (Fig. 5). The identity of this product was confirmed by MALDI-TOF-MS in the negative-ion mode. The substrate, DHPS, gave a peak ($m/z=155.13=[M-H]^{-1}$), which supported the identity of the compound ($M_{\text{calcd}}=156.18$). After addition of enzyme, a product was formed whose signal ($m/z=169.13=[M-H]^{-1}$) confirmed the tentative identification of the product being produced in Fig. 5 as sulfolactate ($M_{\text{calcd}}=170.15$).

The $K_m^{\text{app}} [V_{\text{max}}]$ value for NAD^+ was 160 μM [95 mkat (kg protein) $^{-1}$]. The K_m^{app} value for DHPS was 460 μM . Maximal activity could be detected at pH values between 9 and 10, above which no measurements were done. The

activity decreased rapidly with decreasing pH; at pH 6.5 the enzyme was almost inactive.

Inducible transcription of *hps* genes

HpsNOP were all inducible in *C. pinatubonensis* (see above). Data from RT-PCR experiments (Fig. 6) showed that not only *hpsNOP* but also *hpsU* were transcribed inducibly. As these four genes (*hpsNOUP*) are contiguous, the transcription of all four is unsurprising.

The enzymic data showed that HpsNOP were inducible in *Roseovarius nubinhibens*. RT-PCR experiments showed no transcription (*hpsNP*) or weak transcription (*hpsO*) in acetate-grown cells, whereas DHPS-grown cells showed strong transcription of *hpsNOP*. This was consistent with the enzymic data. In contrast, the putative transporter genes (*hpsKLM*) were apparently constitutively transcribed, whereby *hpsM* gave a strong signal and *hpsKL* gave weak ones. In contrast to *C. pinatubonensis*, the transporter genes are encoded at a distance from *hpsRNOP*, so a difference in regulation of the two clusters is possible.

DISCUSSION

A wide range of three-carbon sulfonates (C_3 -sulfonates) has been observed to derive from sulfoquinovose (e.g. Roy *et al.*, 2003; Shibuya *et al.*, 1963), and these C_3 compounds are often interconvertible (e.g. Cook *et al.*, 2006; Weinstein & Griffith, 1988). However, only (*S*)-cysteate seems to be readily available commercially, and other compounds have to be synthesized by the researcher, often with great difficulty. Our attempts to synthesize DHPS as published (Roy *et al.*, 2003) gave a 6 % yield after a week, so the overnight production of the barium bispropane-2,3-diolsulfonate in the 100 g scale, and later the sodium salt, allowed us to do the present work comfortably. The general method might be old-fashioned (Fries, 1938) but it generates a high yield of high-quality product (Fig. 2).

DHPS, known to be widespread (see Introduction), is degraded by bacteria from marine and terrestrial habitats (Table 1), representative of a much larger group of organisms (Supplementary Table S1). All of these 28 organisms (Supplementary Table S1) share *hpsNOP*, from which we hypothesized their ability to degrade DHPS. Most of them encode the putative transcriptional regulator, HpsR (Supplementary Table S1). The degradative organisms employ three different desulfonation reactions (Table 1), which suffice for the whole group of organisms (Supplementary Table S1). The data indicate at least four different transporters. *C. pinatubonensis* transcribes an MFS-type transport system (Supplementary Table S1), which we find to be rare in BLAST analyses, in that the nearest orthologues (<67 % identity) are IseU, responsible for isethionate uptake (Weinitschke *et al.*, 2010). The most common putative transporter is HpsKLM, but many organisms have no *hpsKLM* genes (Supplementary Table S1).

Fig. 4. Electropherogram (SDS-PAGE) of fractions taken during the purification of HpsN from *C. pinatubonensis* JMP134. Inducible SuyB is marked by the arrow. Lane 1, molecular mass markers (kDa); lane 2, soluble fraction of DHPS-grown cells; lane 3, active fraction after anion-exchange chromatography; lane 4, active fraction after hydrophobic interaction chromatography; lane 5, purified enzyme (97 %) after gel filtration chromatography.

Table 2. Purification table for the NAD⁺-dependent DHPS dehydrogenase HpsN from *C. pinatubonensis* JMP134

Purification step	Protein (mg)	Specific activity (mkat kg ⁻¹)	Activity (nmol s ⁻¹)	Yield (%)	Purification (-fold)
Crude extract	30	3.2	96	100	1
Soluble fraction	25	4.9	123	128	1.5
Anion-exchange chromatography	1.3	93	121	126	29
Hydrophobic interaction chromatography	0.11	84	9.2	10	26
Gel filtration chromatography	0.02	79	1.6	2	25

However, a representative of this group, *Roseobacter denitrificans*, grows well with DHPS (Table 1), so an unknown transporter must be present. Further, the fact that *Ruegeria pomeroyi* degrades DHPS to only 50% (Table 1) might imply that only one, enantiomer-specific DHPS-transporter is present (HpsKLM), and that this and other organisms have another, unknown transporter. Finally, *Verminephrobacter eiseniae* potentially encodes a four-component ABC-type transporter (HpsABB'C) adjacent to *hpsR* and *hpsNOP* (Supplementary Table S1). Thus, with several transporters and desulfonation mechanisms (Table 1), there is considerable biodiversity in DHPS degradation.

The enzymes HpsNOP are often annotated in databases as 'histidinol dehydrogenase', 'gluconate 5-dehydrogenase' and 'L-threonine 3-dehydrogenase', respectively (e.g. in *C. pinatubonensis* JMP134). While these names are somewhat misleading, they indicate that two enzymes could oxidize a secondary alcohol, whereas one oxidizes a primary alcohol. We thus predicted that HpsN would oxidize the primary alcohol of one enantiomer of DHPS, presumably (R)-DHPS, to generate (R)-sulfolactate, the recently recognized enantiomer that is the substrate for SuyAB (Denger & Cook, 2010). And HpsN does, indeed, generate sulfolactate, presumably (R)-sulfolactate, from DHPS (Fig. 5) in an NAD⁺-coupled reaction. Both HpsO (NADP⁺-coupled)

and HpsP (NAD⁺-coupled) oxidize DHPS (Fig. 3), presumably at the secondary alcohol (see above), and presumably with opposite enantiomers of DHPS to effect a racemase reaction. This is analogous to the effective sulfolactate racemase catalysed by two dehydrogenases in many organisms (Denger & Cook, 2010). Given that HpsP is annotated to act on (2S,3R)-threonine, whereby the hydroxyl group at the C3 position has the (R)-configuration, we tentatively suggest that HpsP is (R)-DHPS 2-dehydrogenase and that HpsO is (S)-DHPS 2-dehydrogenase.

We thus deduce that HpsN catalyses the NAD⁺-coupled oxidation of (R)-DHPS to (R)-sulfolactate and that this enzyme should have the systematic name (R)-2,3-dihydroxypropanesulfonate:NAD⁺ 3-oxidoreductase [EC 1.1.1.-]. The corresponding accepted name could be sulfofopropanediol 3-dehydrogenase. Similarly, if the deductions are correct, HpsO would be (S)-sulfofopropanediol 2-dehydrogenase and HpsP would be (R)-sulfofopropanediol 2-dehydrogenase.

Roy *et al.* (2003) established that SQ can be degraded via sulfolactate (and/or equivalent pathways) to DHPS by the bacterium *Klebsiella* sp. strain ABR11, which is a poor degrader of DHPS, and which excretes it quantitatively. We hypothesize that an organism like *C. pinatubonensis* JMP134 would degrade this DHPS by the pathway in Fig. 1. In the marine environment, diatoms and algae generate DHPS from SQ (e.g. Shibuya *et al.*, 1963), and we presume that members of the widespread *Roseobacter* clade can degrade the compound, as do *Roseovarius nubinihibens* ISM and *Ruegeria pomeroyi* DSS-3 (Table 1). We know neither the role of excreted DHPS nor the amounts or fluxes of the compound. Perhaps HpsN could be used to quantify DHPS in native environments.

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Fig. 5. Formation of sulfolactate from DHPS by HpsN from *C. pinatubonensis* JMP134. No sulfolactate was formed in control experiments without DHPS or with heat-inactivated protein.

Fig. 6. Representative agarose gels from RT-PCR experiments: transcripts of *hpsNOPU* from *C. pinatubonensis* JMP134. M, molecular markers (bp); D, RT-PCR with RNA isolated from DHPS-grown cells; A, RT-PCR with RNA isolated from acetate-grown cells; –, negative control omitting template; +, positive control with genomic DNA.

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