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Metabolism of 2,3-dihydroxypropane-1-sulfonate by marine bacteria†

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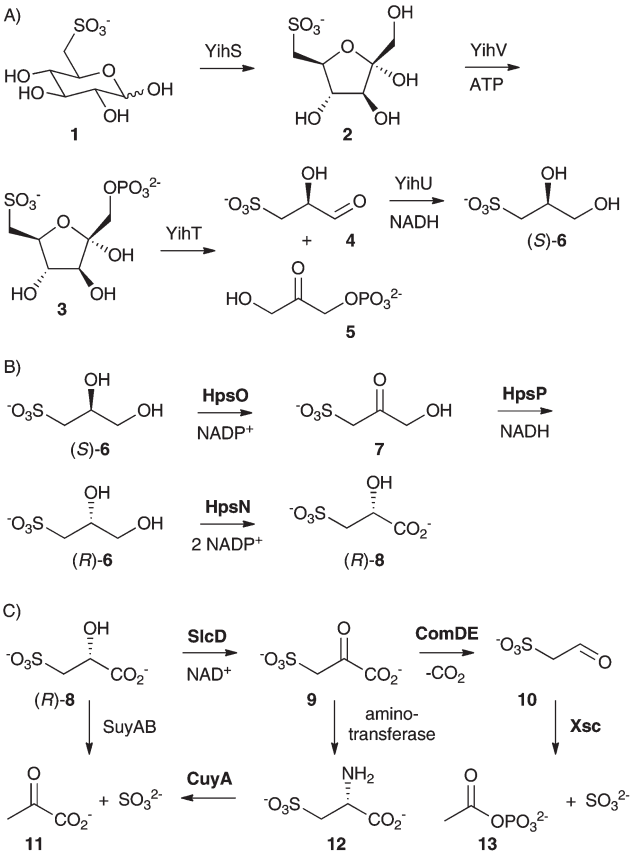
Both enantiomers of the sulfoquinovose breakdown product 2,3-dihydroxypropane-1-sulfonate, an important sulfur metabolite produced by marine algae, were synthesised in a ^{34}S-labelled form and used in feeding experiments with marine bacteria. The labelling was efficiently incorporated into the sulfur-containing antibiotic tropodithietic acid and sulfur volatiles by the algal symbiont *Phaeobacter inhibens*, but not into sulfur volatiles released by marine bacteria associated with crustaceans. The ecological implications and the relevance of these findings for the global sulfur cycle are discussed.

Sulfoquinovose (**1**) is a sugar that occurs in sulfoquinovosyl diacylglycerol (SQDG) sulfolipids that were discovered more than 50 years ago[1] and are today known to generally occur in higher plants and algae, and most photosynthetic bacteria.[2–4] The estimated annual production of 10^{10} t (ref. 5) makes **1** besides methionine, cysteine, glutathione and dimethylsulfoniopropionate (DMSP)[6] one of the most abundant organic sulfur compounds in nature. The bacterial degradation of **1** to 2,3-dihydroxypropane-1-sulfonate (**6**, DHPS) was initially suggested to proceed *via* glycolysis,[7] but recently a specific catabolon with similar intermediates as in glycolysis was discovered (Scheme 1A).[8] The absolute configurations of the pathway intermediates were not specified in the original report, but assuming that all stereocentres in the catabolites of **1** are retained, the pathway will end at (*S*)-**6**. This metabolite is also a major constituent in the cytosol and massively excreted by the diatom *Thalassiosira pseudonana*,[9] an organism that lives in symbiosis with bacteria from the *Roseobacter* group by providing DHPS, while the alga depends on exogenous vitamin B12 excreted by the bacteria.[10] The expression of bacterial genes for uptake and catabolism of DHPS is strongly upregulated in cocultures of the diatom and its bacterial symbionts.[9] Therefore, DHPS is a potential source of carbon and sulfur for



Scheme 1 Sulfoquinovose (**1**) and DHPS catabolism. (A) Degradation of **1** to DHPS (*S*)-**6** by *E. coli*, (B) conversion of (*S*)-**6** to (*R*)-**8**, and (C) alternative catabolic pathways for the degradation of (*R*)-**8** to sulfite. Enzymes shown in bold are encoded in *P. inhibens* DSM 17395.

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†Electronic supplementary information (ESI) available: Synthetic procedures, isolation and identification of new bacterial strains, analytical data from feeding experiments with (^{34}S)DHPS, information about genes for DHPS catabolism in *P. inhibens*, and NMR spectra of synthetic compounds. See DOI: 10.1039/c7ob00357a

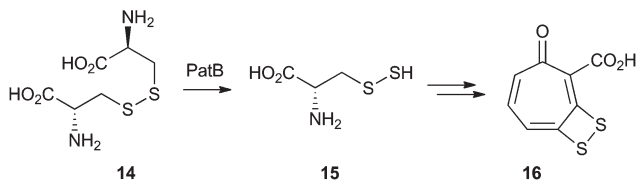
marine bacteria that are capable of DHPS uptake and catabolism.

DHPS degradation makes use of the enzymes encoded in the *hps* gene cluster[11,12] that spans genes for the transcriptional regulator HpsR, the major facilitator superfamily transporter HpsU, the dehydrogenases HpsO, HpsP and HpsN, and the (*R*)-sulfolactate sulfo-lyase SuyAB.[13,14] The end product of the sulfoquinovose degradation (*S*)-**6** may be epimerised by HpsO and HpsP *via* 2-oxo-3-hydroxypropane-1-sulfonate (**7**) to (*R*)-**6** (Scheme 1B), followed by HpsN oxidation to 3-sulfolactate (**8**) with an unknown absolute configuration,[12] however SuyAB requires the substrate (*R*)-**8** that reflects likely the enantiomer provided by HpsN. Besides the cleavage to pyruvate (**11**) and SO_3^{2-} by SuyAB[13,14] a few other pathways for the further degradation of (*R*)-**8** are known (Scheme 1C), including oxidation to sulfopyruvate (**9**) by SlcD,[15] transamination to L-cysteate (**12**) by an unidentified enzyme[16] and desulfonation to **11** by the PLP-dependent CuyA.[17] Decarboxylation to sulfoacetaldehyde (**10**) by ComDE followed by desulfonation by Xsc yields acetyl phosphate (**13**) that enters the C_2 pool and is used as a carbon source.[15,18,19] The sulfite may simply be exported by the specific exporter TauE,[20] but could also be a source for other sulfur metabolites. We show here by isotopic labelling experiments that the DHPS sulfur is efficiently used for the biosynthesis of secondary metabolites and sulfur volatiles specifically by marine bacteria associated with algae.

A good candidate organism for our investigations is *Phaeobacter inhibens* DSM 17395, a marine bacterium of the *Roseobacter* group (family Rhodobacteraceae) that lives in symbiosis with marine algae and that is known to produce various sulfur metabolites.[21,22] In the symbiotic relationship with the microalga *Emiliania huxleyi* the compatible solute dimethylsulfoniopropionate (DMSP)[23] is supplied as a carbon and sulfur source to the bacterium,[24] and, in return, *P. inhibens* produces the growth promoter phenylacetic acid and the proton antiporter[25] tropodithietic acid (TDA, **16**) that has antibiotic activity[21] and protects the haptophyte from bacterial infections.[21] The biosynthesis of TDA depends on the TDA biosynthetic gene cluster[26] and a biosynthetic model for the incorporation of sulfur into TDA proceeds *via* *S*-thiocysteine (**15**) that can arise from cystine (**14**) *via* cleavage by PatB (Scheme 2),[27,28] an enzyme that has homology to cystathionine β-lyases. This model was supported by the incorporation of labelling from $^{34}SO_4^{2-}$ and, with much higher rates, from (^{34}S)cysteine and ($^{34}S_2$)cystine.[27] We have also previously reported about the incorporation of labelling from (methyl-$^2H_6$)DMSP and (^{34}S)DMSP into sulfur volatiles such as dimethyl disulfide and dimethyl trisulfide by *P. inhibens*,[22,29,30] which is consistent with an efficient degradation *via* the DMSP demethylation pathway that ends at methanethiol.[31] As an extension of this work the use of DHPS by *P. inhibens* as a sulfur source for secondary metabolites is addressed here.

For the investigation of biosynthetic pathways feeding experiments with isotopically labelled compounds are useful.[32] Therefore, racemic (^{34}S)DHPS (**6**) was synthesised starting from solketal (**17**) that was converted into the mesylate **18** (Scheme 3). Nucleophilic substitution with K^{34}SCN[31] yielded the thiocyanate **19** that was reduced to the thiol **20** with $LiAlH_4$. Its oxidation with H_2O_2 proceeded with simultaneous loss of the protecting group to yield (^{34}S)DHPS with 24% yield *via* four steps.

P. inhibens DSM 17395 liquid cultures release the sulfur volatile dimethyl disulfide (DMDS), its oxidation product *S*-methyl methanethiosulfonate and dimethyl trisulfide (DMTS), and small amounts of dimethyl tetrasulfide (Fig. 1 of the ESI†). Synthetic (*rac*)-(^{34}S)DHPS was fed to a *P. inhibens* liquid culture and the volatiles emitted by this strain were trapped on a charcoal filter by the use of a closed-loop stripping apparatus (CLSA).[33] Filter extracts with CH_2Cl_2 were analysed by GC/MS, showing the incorporation of ^{34}S-labelling into dimethyl trisulfide and dimethyl tetrasulfide, but not into dimethyl disulfide and *S*-methyl methanethiosulfonate (Fig. 1



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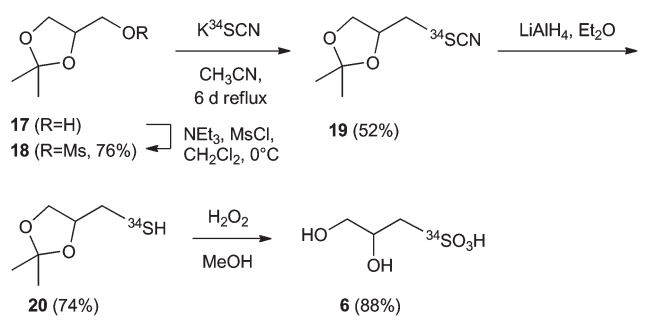
Scheme 2 TDA and its biosynthesis from cystine *via* *S*-thiocysteine.



Scheme 3 Synthesis of (^{34}S)DHPS.

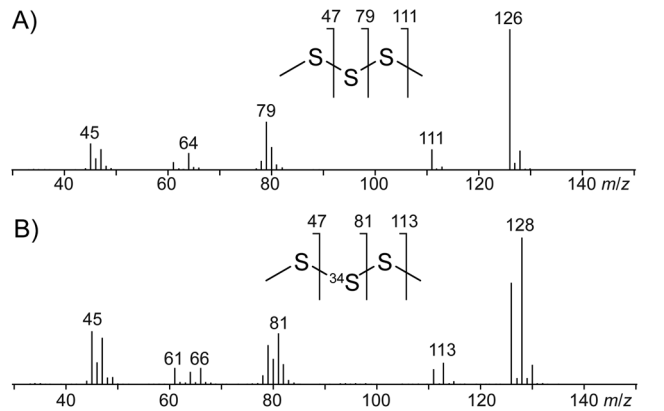


Fig. 1 EI mass spectra of (A) dimethyl trisulfide and (B) labelled DMTS obtained after feeding of (*rac*)-(^{34}S)DHPS.

and Fig. 1 of the ESI†). Careful interpretation of the fragmentation pattern located the incorporated ^{34}S-labelling at the central (non-methylated) sulfur(s) of DMTS (55% incorporation rate for the central sulfur) and dimethyl tetrasulfide (Fig. 1 of the ESI†) by an increase of those fragment ions that contain one of the central sulfurs (m/z = 79 and m/z = 111), but not of the fragment ion representing a MeS group (m/z = 47).

To investigate which of the two enantiomers of **6** serves as a precursor for DMTS both (*R*)- and (*S*)-(^{34}S)DHPS were synthesised *via* the route shown in Scheme 3 from the commercially available pure enantiomers of **17**. Both compounds were obtained in high enantiomeric purity (Fig. 2 of the ESI†). Feeding of (*R*)- and (*S*)-(^{34}S)DHPS to *P. inhibens* resulted in the incorporation into the central sulfur atom of DMTS for both enantiomers (Fig. 3 of the ESI†), with incorporation rates of 10% from (*R*)-(^{34}S)DHPS and 30% from (*S*)-(^{34}S)DHPS. This finding is explainable by the formation of hydrogen sulfide from DHPS as a source of the central sulfur of DMTS, while the methylated sulfurs may directly arise from methionine degradation to methanethiol. The reaction of two units of methanethiol with one equivalent of hydrogen sulfide under oxidative conditions may then result in DMTS. A clear incorporation of labelling was also observed in both sulfur atoms of TDA as indicated by increased ions at $[M + 2]^+$ and $[M + 4]^+$ from both enantiomers of DHPS (9% incorporation rate from (*S*)-(^{34}S)DHPS and 24% from (*R*)-(^{34}S)DHPS, Fig. 4 of the ESI†). These data indicate that both enantiomers of DHPS are taken up by *P. inhibens* and can be used as a sulfur source for sulfur volatiles and TDA.

A detailed analysis of the genome of *P. inhibens* reveals the presence of several genes for the degradation of DHPS (Fig. 2 and Table 2 of the ESI†). This includes the *hps* gene cluster composed of genes for the regulator HpsR (black), for a tripartite transport system (green) presumably for DHPS uptake, for the dehydrogenases, HpsNOP, and directly adjacent genes for a universal stress protein (purple), for the sulfopyruvate decarboxylase ComDE, and the sulfolactate dehydrogenase SlcD. Upstream of this cluster a standalone gene for the PLP-dependent L-cysteate sulfolyase CuyA and, further upstream, a cluster of genes for a transcriptional regulator (black), the sulfoacetaldehyde acetyltransferase Xsc, for the sulfite exporter TauE (green),[20] and for an acetate kinase and a phosphate acetyltransferase (blue) for export and further conversion of the Xsc products sulfite and acetyl phosphate (**13**) are found.

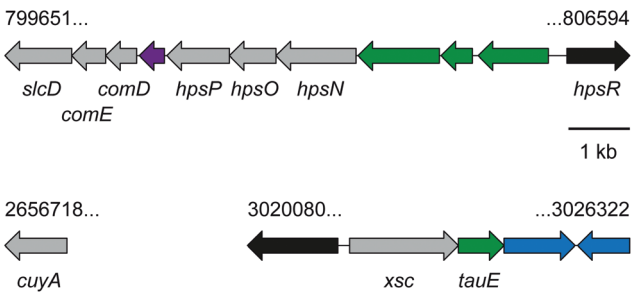


Fig. 2 Genes for DHPS degradation in *P. inhibens* DSM 17395. Gray: directly involved in DHPS degradation, green: transporters, black: transcriptional regulators, purple: universal stress protein, blue: further conversion of acetyl phosphate. Numbers above arrows indicate nucleotide positions of the borders of the gene clusters.

This genetic information demonstrates that *P. inhibens* can convert both enantiomers of DHPS *via* the pathways shown in Scheme 1, yielding sulfite from **9** *via* degradation by ComDE and Xsc and possibly also *via* transamination and cleavage by CuyA. Sulfite may either be exported, but can also be used for the biosynthesis of sulfur metabolites by reduction to sulfide *via* the sulfite reductase that is encoded in *P. inhibens* (accession number WP_014880398) and incorporation into cysteine that was previously shown to be a good precursor both for sulfur volatiles and TDA.[27,29]

The link between the uptake of the algal sulfoquinovose catabolite DHPS and TDA biosynthesis in *P. inhibens* is of ecological importance, because *P. inhibens* colonises marine algae such as *Emiliania huxleyi* and *Ulva australis* and protects the plant host by excreting its antibiotic TDA.[34] Recent research by the Moran group has shown that DHPS excreted by marine algae is eagerly taken up by their bacterial symbionts. We have unraveled here additional functions of DHPS in the symbiotic interaction between algae and bacteria, because the sulfur in this compound is used to produce the sulfur containing antibiotic TDA for algal protection. To further investigate the specificity of our findings five other marine bacteria, isolated from the carapace of the crustacean *Cancer pagurus* and affiliated with the family Rhodobacteraceae (Table 1 of the ESI†), were investigated for the incorporation of labelling from (*rac*)-(^{34}S)DHPS into DMTS, a sulfur volatile consistently found in the headspace extracts of all bacterial isolates. As a positive control, (^{34}S)cysteine was fed in the second series of experiments. In all cases no incorporation of the sulfur labelling from (*rac*)-(^{34}S)DHPS could be observed, while the labelling from (^{34}S)cysteine was efficiently taken up into the central sulfur of DMTS (50–90% incorporation rate, for strain CP127 an additional 15% incorporation was observed into the MeS groups, Fig. 5 of the ESI†). These observations reflect a highly specific adaption of marine bacteria such as *P. inhibens* to their algal symbionts, while other marine bacteria associated with crustaceans lack the capacity for the degradation of DHPS, a metabolite that is not produced by animals. Because of the large amounts of DHPS produced in the oceans and the high abundance of this compound especially in phytoplankton blooms[9] the conversion of DHPS into sulfur volatiles by bacteria associated with algae has ample effects and is likely of similar importance for the global sulfur cycle as the well investigated bacterial DMSP degradation to volatile sulfur compounds.[31,35–37]

Conclusions

P. inhibens and some other bacteria of the *Roseobacter* group are well known for strong interactions with their hosts.[24]

Specific adaptations are also reflected by the results of our study and are in accordance with the isolation source. Several strains of *P. inhibens* that were obtained from different hosts were shown to be metabolically extremely versatile and capable of living with various eukaryotic organisms including algae in previous work.[38] Besides the algal sulfur metabolite DMSP, we have shown here that the sulfur metabolite DHPS, an algal breakdown product of sulfoquinovose, is taken up and used for the biosynthesis of the sulfur containing antibiotic TDA and sulfur volatiles by *P. inhibens*. The new strains from the carapace of *C. pagurus* appear not to have the potential to use DHPS which underpins the specificity of the adaption of *P. inhibens* to its algal symbiont.

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