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Pathways and Substrate Specificity of DMSP Catabolism in Marine Bacteria of the *Roseobacter* Clade

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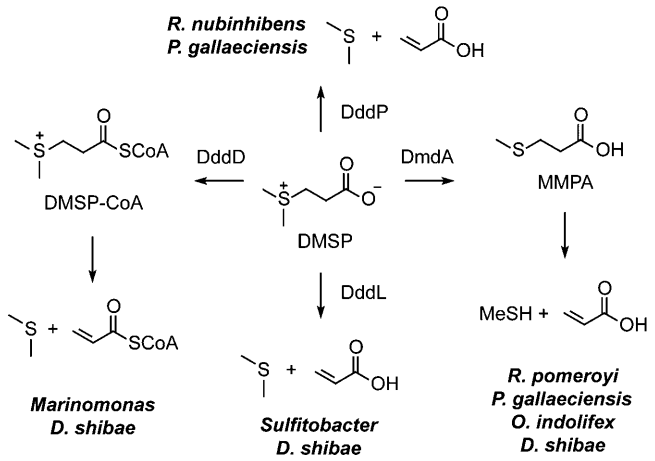
The volatiles released by *Phaeobacter gallaeciensis*, *Oceanibulbus indolifex* and *Dinoroseobacter shibae* have been investigated by GC-MS, and several MeSH-derived sulfur volatiles have been identified. An important sulfur source in the oceans is the algal metabolite dimethylsulfoniopropionate (DMSP). Labelled [$^2H_6$]DMSP was fed to the bacteria to investigate the production of volatiles from this compound through the lysis pathway to [$^2H_6$]dimethylsulfide or the demethylation pathway to [$^2H_3$]-3-(methylmercapto)propionic acid and lysis to [$^2H_3$]MeSH. [$^2H_6$]DMSP was efficiently converted to [$^2H_3$]MeSH by all three species. Several DMSP derivatives were synthesised and used in feeding experiments. Strong dealkylation activity was observed for the methylated ethyl methyl sulfoniopropionate and dimethylseleniopropionate, as indicated by the formation of EtSH- and MeSeH-derived volatiles, whereas no volatiles were formed from dimethyltelluriopropionate. In contrast, the dealkylation activity for diethylsulfoniopropionate was strongly reduced, resulting in only small amounts of EtSH-derived volatiles accompanied by diethyl sulfide in *P. gallaeciensis* and *O. indolifex*, while *D. shibae* produced the related oxidation product diethyl sulfone. The formation of diethyl sulfide and diethyl sulfone requires the lysis pathway, which is not active for [$^2H_6$]DMSP. These observations can be explained by a shifted distribution between the two competing pathways due to a blocked dealkylation of ethylated substrates.

Introduction

The *Roseobacter* clade forms one of the most important groups of marine microorganisms.[1] They are distributed from polar to tropical regions, are present in coastal and open-ocean environments and are often associated with algae.[2] The main sulfur metabolite produced by algae in high intracellular concentrations, and the main source of marine organic sulfur, is dimethylsulfoniopropionate (DMSP).[3] Large amounts of DMSP are degraded by marine bacteria according to two competing pathways, that is, lysis to dimethyl sulfide (DMS) by a DMSP lyase or demethylation to 3-(methylmercapto)propionic acid (MMPA, Scheme 1). Downstream processing of MMPA includes the elimination of MeSH.[4]



Scheme 1. DMSP degradation pathways. DMSP can be transformed into its CoA ester (DMSP-CoA) by the acyl-CoA transferase DddD and subsequently cleaved to dimethyl sulfide (DMS) and acryloyl-CoA, lytically cleaved to DMS and acrylate by the DMSP lyase DddL, or degraded to DMS and acrylate by an unknown mechanism involving the DMSP lyase DddP. Alternatively, DMSP can be degraded on the demethylation pathway to 3-(methylmercapto)propionic acid (MMPA) by the DMSP demethylase DmdA, that may be transformed into methanethiol (MeSH) and acrylate by an unknown enzyme.

Several lysis mechanisms have been identified. DMSP can be transformed to its CoA ester, which is cleaved to DMS and acryloyl-CoA in a process catalysed by the acyl-CoA transferase DddD first identified in *Marinomonas*.[5] Alternatively, in *Sulfitobacter*, the DMSP lyase DddL catalyses the formation of DMS and acrylate from DMSP.[6] The recently identified DMSP lyase DddP from *Roseovarius nubinhibens* cleaves DMSP to DMS by an unknown mechanism.[7] The DMSP demethylase DmdA was first described from *Ruegeria pomeroyi*.[8] No genes or enzymes for the lysis of MMPA to MeSH are known.

DMS is important in the biogeochemical sulfur cycle,[9] in which SO_4^{2-} is transported from the continents via the rivers to the oceans, while the backflow to land requires the participation of sulfur gases. The microbial lysis of DMSP to DMS causes an estimated annual sulfur flux from the oceans to the atmosphere of 13 to 45 million tonnes of sulfur.[10] Atmospheric DMS is rapidly oxidised to methanesulfonic acid and SO_4^{2-}, which rains down to earth to close the cycle. The massive bac-

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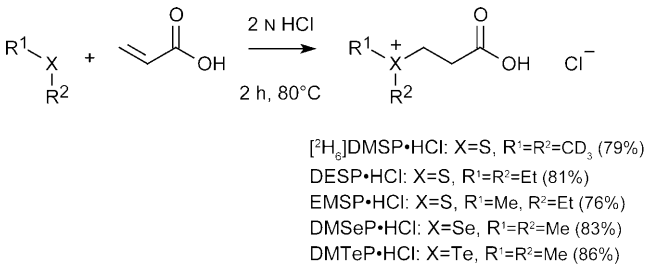
terial production of DMS is of climatical relevance, because atmospheric SO_4^{2-} initiates the formation of aerosol particles that act as cloud-condensation nuclei. The resulting clouds control the reflectance, scattering and absorbance of sunlight and directly influence the global radiation budget.[11] High DMS flux rates consequently have a cooling effect and lead to slower growth of the algae, thus limiting DMS production.[12] The demethylation pathway also limits sulfur flux, because MeSH is highly soluble in water and reacts to form metal–MeSH complexes, with an impact on the availability of trace metals in seawater.[13] The majority of DMSP seems to be demethylated by marine bacteria, whereas only a minor portion is cleaved to DMS; this suggests that its release from the oceans is tightly controlled by the bacterioplankton community.[14]

This work focuses on three species of the *Roseobacter* clade with sequenced genomes: *Phaeobacter gallaeciensis* DSM 17395^T, *Oceanibulbus indolifex* DSM 14862^T and *Dinoroseobacter shibae* DSM 16493^T (http://img.jgi.doe.gov/).[15] Recently, the volatiles released by liquid cultures of two of these strains, *P. gallaeciensis* and *O. indolifex*, have been reported,[16] with sulfur volatiles forming the main compound class present in the headspace extracts. Here we report on the volatiles released by these two species and *D. shibae* from agar plate cultures and fed with DMSP as an important source of organic sulfur available to bacteria of the *Roseobacter* clade in the marine environment. Several DMSP derivatives were fed to the bacteria, resulting in the production of a variety of novel volatiles. As will be demonstrated in this article, these feeding experiments also gave insight into the distribution between the two DMSP catabolic pathways and served to test the specificities of the encoded DMSP-degrading enzymes in vivo.

Results and Discussion

Genome analysis, synthesis of the hydrochlorides of [$^2H_6$]DMSP and DMSP derivatives, and analytical methods

A BLAST search revealed that *P. gallaeciensis* encodes a DMSP lyase with high homology to DddP, and *D. shibae* encodes DddD and DddL homologues. None of the known DMSP lyases is present in *O. indolifex*, whereas all three organisms encode DmdA homologues (Scheme 1). To test the biosynthetic capacities of these species to produce sulfur volatiles from DMSP, the hydrochloride of isotopically labelled [$^2H_6$]DMSP was synthesised by the acid-catalysed (HCl) addition of [$^2H_6$]DMS to prop-2-enoic acid (Scheme 2) and fed to agar plate cultures of the bacteria. Similarly, the hydrochlorides of the DMSP derivatives diethylsulfoniopropionate (DESP), ethyl methyl sulfoniopropionate (EMSP), dimethylseleniopropionate (DMSeP) and dimethyltelluriopropionate (DMTeP) were synthesised and fed to agar plate cultures of the bacteria to investigate the substrate specificities of the involved enzymes in vivo. In all the feeding experiments described in this article, the hydrochlorides of [$^2H_6$]DMSP and its derivatives were used, but for simplicity and brevity the terms [$^2H_6$]DMSP, DESP, EMSP, DMSeP and DMTeP will be used. The volatiles released by the bacterial cultures in these feeding experiments were collected by use of the closed-loop stripping apparatus (CLSA) technique, and the obtained extracts were analysed by GC-MS.[17] To analyse the highly volatile compounds covered by the solvent peak of the CLSA headspace extracts, the feeding experiments with [$^2H_6$]DMSP were repeated but with the solvent-free, solid-phase microextraction (SPME) technique.



Scheme 2. Synthesis of the hydrochlorides of [$^2H_6$]DMSP and derivatives.

Volatiles from *Phaeobacter gallaeciensis* DSM 17395^T

The volatiles released by agar plate cultures of *P. gallaeciensis* are summarised in Table S1 of the Supporting Information, and the gas chromatogram is shown in Figure 2, below. As with the recently reported headspace composition of *P. gallaeciensis* grown in liquid medium,[16] several sulfur compounds including dimethyl disulfide (**2**), dimethyl trisulfide (**5**), *S*-methyl methanethiosulfonate (**12**) and *S*-methyl propanethioate (**23**) were produced, in addition to ethyl benzoate (**29**, Figure 1). All identified sulfur volatiles contained methylthio groups and were derived from MeSH by known reactions, such as oxidation with molecular oxygen in the presence of ascorbate and transition metal ions or charcoal as used in the CLSA filters, nucleophilic substitution (to **2**, **5**, and **12**), or transesterification of fatty acyl-CoA derivatives (to **23**, Scheme 3).[18]

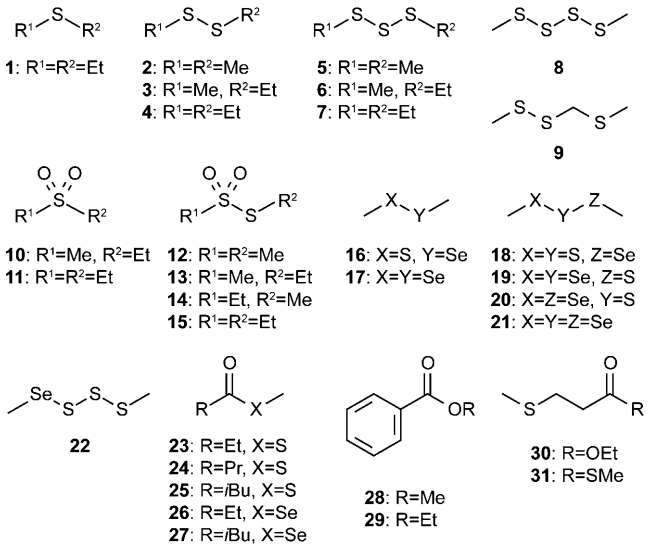
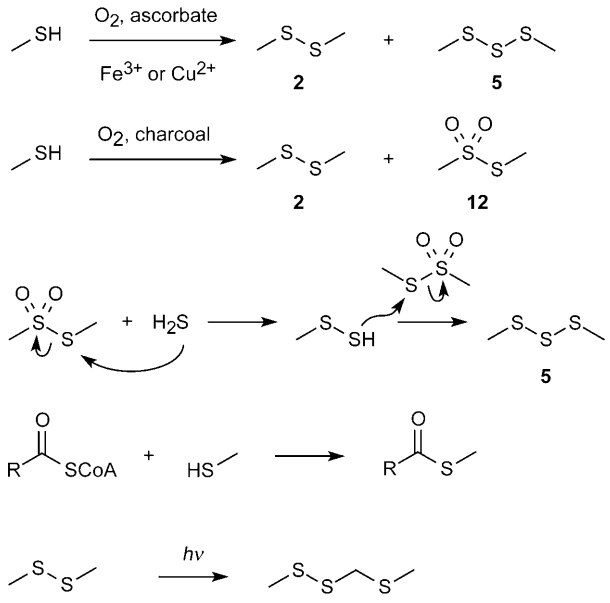


Figure 1. Volatiles from bacteria of the *Roseobacter* clade.



Scheme 3. Known reactions to sulfur volatiles from MeSH.

Feeding of [$^2H_6$]DMSP to *P. gallaeciensis* led to rapid degradation to [$^2H_3$]MMPA catalysed by DmdA and possibly by further as yet unidentified DMSP demethylases. Subsequently [$^2H_3$]MMPA was cleaved to [$^2H_3$]MeSH. The incorporation of labelling into MeSH-derived volatiles **2**, **5** and **12** proceeded with high rates ($\geq$90%, Table S1). These high incorporation rates demonstrated that the majority of MeSH-derived molecules were produced from [$^2H_6$]DMSP and not from alternative sulfur sources in the medium. Deuterated compounds elute with shorter GC retention times than their unlabelled counterparts because of the shorter C–D versus C–H bond length; this results in a lower polarisability and a weaker (induced) dipole interaction of the analyte with the stationary phase.[19] Even if several isotopomers with a different deuterium content are present in one sample, they can be separated. The mass spectra of all chromatographically separated isotopomers of **2**, **5** and **12** are shown in Figure S1.

Feeding of the DMSP derivatives DESP, EMSP, DMSeP and DMTeP resulted in all cases in the production of **2**, **5** and **12** obviously originating from sulfur sources such as sulfate in the medium. Additionally, in the feeding experiment with DESP, the volatiles **23**, *S*-methyl 3-methylbutanethioate (**25**) and **29**, but only small amounts of the EtSH-derived compound ethylmethyldisulfide (**3**) were found. The formation of **3** from DESP can be explained by the dealkylation activity of DmdA and possible further unidentified DMSP demethylase(s) to yield 3-(ethylmercapto)propionic acid (EMPA), which is cleaved to EtSH. At least one of the two steps is comparably slow to the equivalent reactions on DMSP or MMPA. A reduced activity towards DESP compared to DMSP in the dealkylation of one *S*-alkyl group is likely to be more significant than reduced activity towards EMPA compared to MMPA in the lysis to the respective alkanethiol, either due to a higher steric hindrance of the *S*-ethyl group compared to the *S*-methyl group in its nucleophilic attack, or due to the higher overall steric hindrance of the DESP substrate compared to DMSP. In conclusion, the DMSP demethylase(s) and the proposed MMPA lyase(s) in *P. gallaeciensis* are able to transform EMSP into EtSH and EtSH-derived volatiles, albeit with significantly reduced turnover rates of the overall pathway.

The feeding experiment with DESP also yielded diethyl sulfide (**1**), covered by **2** (Figure 2), that arose from lysis activity towards DESP, whereas the respective lysis product, [$^2H_6$]DMS, from [$^2H_6$]DMSP could not be detected by the CLSA technique because the highly volatile [$^2H_6$]DMS coelutes with the solvent. To exclude a misinterpretation of the data obtained in the feeding experiment with [$^2H_6$]DMSP, the respective agar plate cultures were reanalysed by using solvent-free SPME. The SPME headspace extract contained [$^2H_6$]-**2**, but no [$^2H_6$]dimethyl sulfide (Figure S2); this supports a DMSP catabolism that strongly favours the demethylation pathway over the lysis pathway. The shifted distribution between these two pathways in the feeding experiment with DESP towards the lysis pathway, expressed by the production of **1**, can be explained by a blocked dealkylation of this substrate that enables a substantial participation of the lysis pathway.

In the feeding experiment with EMSP, the majority of sulfur volatiles were derived from EtSH, represented by **3**, **4**, ethyl methyl trisulfide (**6**), diethyl trisulfide (**7**), *S*-ethyl methanethiosulfonate (**13**), *S*-methyl ethanethiosulfonate (**14**) and *S*-ethyl ethanethiosulfonate (**15**). The identity of thiosulfonates **13**–**15** was established by synthesis from the sulfonyl chlorides and ethanethiol or sodium methanethiolate (Scheme 4, for mass spectra see Figure S3). The formation of mainly EtSH-derived sulfur volatiles from EMSP points to an efficient demethylation to EMPA, the same intermediate as obtained by the de-ethylation of DESP. This observation is in full accordance with the postulated narrow substrate specificity of the enzyme machinery for the dealkylation step, acting only efficiently in the case of a demethylation but not of a de-ethylation step, whereas the rather broad substrate specificity of the proposed MMPA lyase(s) catalysing the lytic cleavage of MMPA and EMPA is supported.

To further test the in vivo substrate specificities of the enzymes of the DMSP degradation pathways, DMSeP was fed to *P. gallaeciensis*. Besides dimethyl tetrasulfide (**8**), large quantities of MeSeH-derived selenium compounds were emitted (for mass spectra see Figure S4). The principle component in the headspace extract was dimethyl diselenide (**17**) accompanied by dimethyl triselenide (**21**). Furthermore, dimethyl selenyl sulfide (**16**), methyl methylseleno disulfide (**18**), methyl methylthio diselenide (**19**), bis(methylseleno)sulfide (**20**) and methyl methylseleno trisulfide (**22**) were found (for compound identification see discussion in the Supporting Information, especially Figure S5). Strikingly, this mixture of polychalcogenides is simply obtained in a chalcogenide-exchange reaction of **5** with **17**;[20] this suggests that the volatiles **18**–**22** are generated non-enzymatically from **5** and **17**.

Another class of selenium compounds comprises the *Se*-methyl alkaneselenoates, represented by *Se*-methyl propaneselenoate (**26**) and *Se*-methyl 3-methylbutaneselenoate (**27**). Their identity has been confirmed by a synthesis from the re-

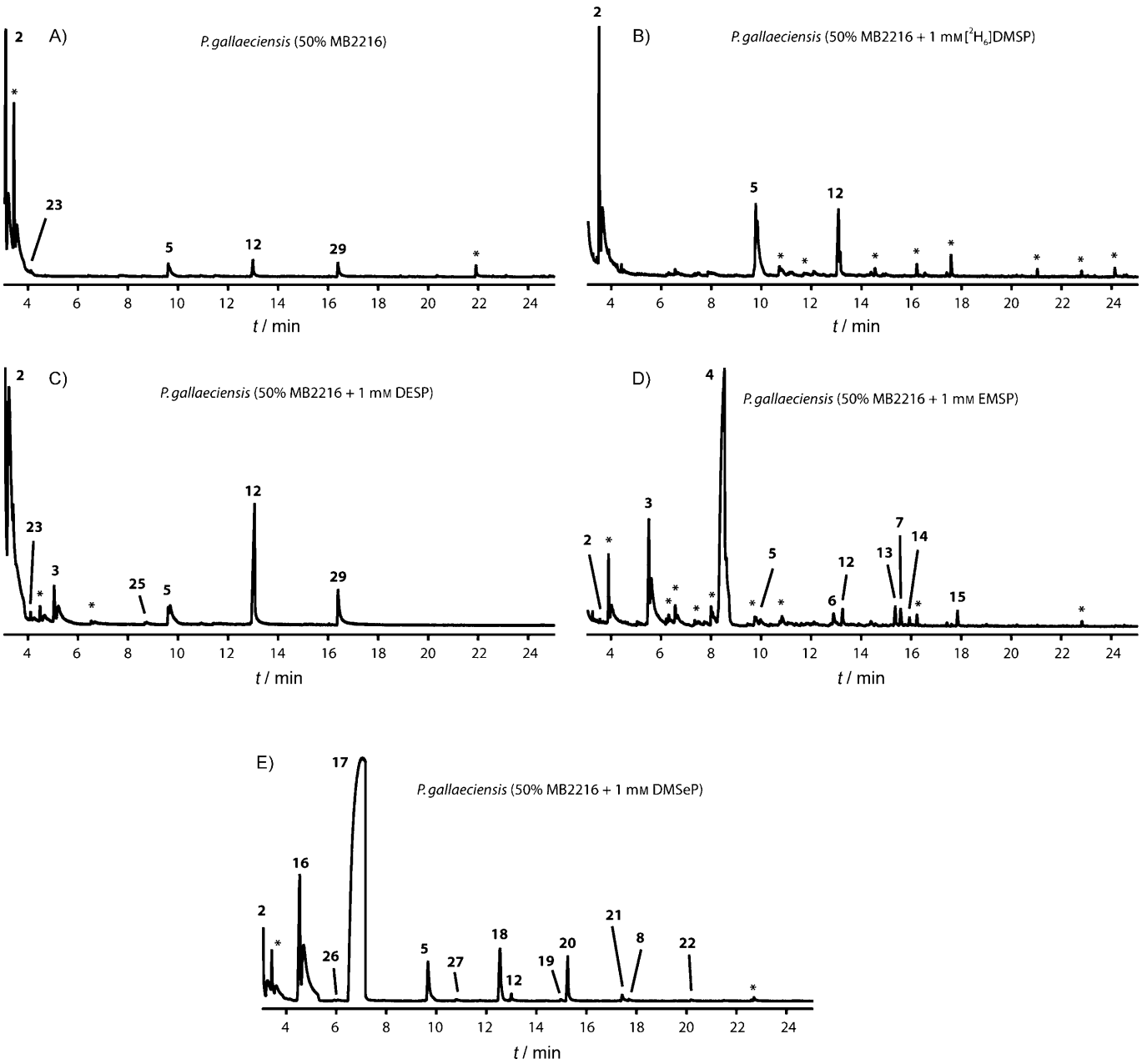
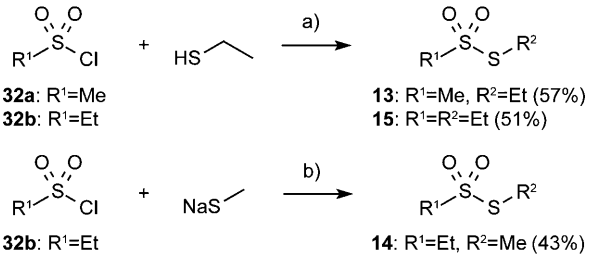


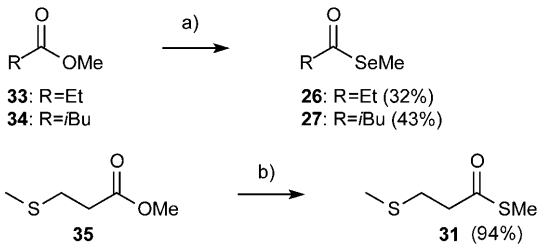
Figure 2. Total ion chromatograms of the CLSA extracts from *P. gallaeciensis* agar plate cultures. The headspace extracts were obtained from A) MB2216 medium, B) MB2216 + 1 mM [$^2H_6$]DMSP·HCl, C) MB2216 + 1 mM DESP·HCl, D) MB2216 + 1 mM EMSP·HCl and E) MB2216 + 1 mM DMSeP·HCl. The peaks are marked with the compound numbers used in the text. Signals arising from the medium, artefacts, and unidentified compounds are labelled with asterisks.



Scheme 4. Synthesis of the thiosulfonates **13**–**15**: a) pyridine, b) Et_2O.

spective methyl esters using dimethyl aluminium methaneselenolate, which is readily available from trimethyl aluminium and selenium (Scheme 5).[21] These compounds were probably formed in a chemical reaction from MeSeH and the corresponding fatty acyl-CoA derivatives.[18]

The presence of all these MeSeH-derived volatiles demonstrated that DMSeP was efficiently demethylated to 3-(methylseleno)propionic acid (MSePA) and lysed to MeSeH by *P. gallaeciensis*, thus showing that the involved enzymes accept and transform the selenium analogue of DMSP. DMSeP occurs natu-



Scheme 5. Synthesis of selenoate and thioate esters: a) Se, Me_3Al, toluene; b) S, Me_3Al, toluene.

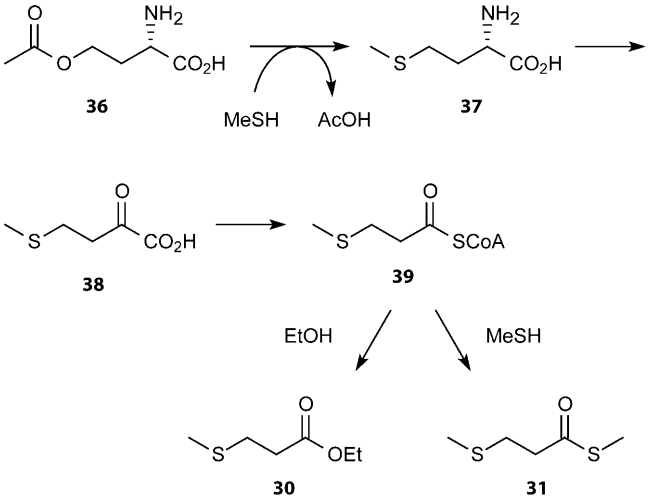
rally as a metabolite produced by the plant *Spartina alternifolia* when amended with toxic SeO_4^{2-} and is an intermediate in its transformation to dimethyl selenide.[22] The DMSP lyases from *Alcaligenes* and *Oceanimonas doudoriffii* have previously been shown to cleave DMSeP to dimethyl selenide and acrylate.[23] Although the demethylation of DMSP and subsequent lysis to MeSH by bacteria is a well-known process, the bacterial demethylation of DMSeP and lysis to MeSeH have not been reported before.

In contrast to DMSeP, the tellurium analogue DMTeP was not transformed by *P. gallaeciensis* to MeTeH, and no tellurium volatiles were found, but only the sulfur volatile **23**. DMTeP might not be taken up by the bacteria, or the enzymes of the DMSP demethylation pathway might not be able to demethylate DMTeP and/or to lyse the respective demethylation product.

In summary these results suggest that the DMSP demethylase DmdA encoded in the genome of *P. gallaeciensis* and possibly additional unidentified DMSP-demethylating enzymes are able to efficiently demethylate DMSP, EMSP and DMSeP, whereas DESP is de-ethylated with a strongly reduced activity. The lysis of the dealkylation products seems to proceed with high activity in all cases, because the dealkylation of EMSP and DESP results in the same proposed intermediate, EMPA. However, the formation of EtSH-derived volatiles from EMSP is efficient, whereas from DESP it is not.

Volatiles from *Oceanibulbus indolifex* DSM 14862^T

A similar set of feeding experiments was carried out with *O. indolifex*. The results closely resembled those from *P. gallaeciensis* (Table S2 and Figure 3). Additional sulfur volatiles from *O. indolifex* not found in *P. gallaeciensis* were methyl methylthiomethyl disulfide (**9**), *S*-methyl butanethioate (**24**), ethyl 3-(methylthio)-propionate (**30**) and *S*-methyl 3-(methylthio)propanethioate (**31**). The identity of **31** was verified by synthesis from methyl 3-(methylthio)propionate (Scheme 5). Feeding of [$^2H_6$]DMSP resulted in the efficient incorporation of deuterium into all sulfur volatiles, thereby demonstrating that DMSP was rapidly converted to MeSH. All the carbons of compound **9** were deuterated (Figure S6); this is in accordance with its reported formation by photolysis of **2** (Scheme 3).[24] The labelling was also introduced into the *S*-methyl groups of the alkanethioates **23**–**25** and of **30**, and into both *S*-methyl groups of **31** (Figure S7), thus pointing to an uptake of [$^2H_3$]MeSH into the amino acid pool (Scheme 6). The volatiles **30** and **31** are likely to be derived from L-methionine that is formed from MeSH and *O*-acetyl homoserine under catalysis of the methionine synthase. Transamination and oxidative decarboxylation lead to 4-(methylmercapto)-2-oxobutyric acid (**38**), which can be transformed into its CoA thioester (**39**), which serves as a precursor for **30** and **31** by formal transesterification with EtOH or MeSH, respectively.



Scheme 6. Biosynthesis of sulfur volatiles from methanethiol via the amino acid pool.

SPME analysis showed that only traces of [$^2H_6$]DMS close to the detection limit were produced in the feeding experiment with [$^2H_6$]DMSP (Figure S2).[25] Feeding of DESP yielded small amounts of **1**, presumably arising from enzymatic lysis. None of the known DMSP lyases, DddD, DddL or DddP, is encoded in the genome of *O. indolifex*. The formation of these lysis products, albeit in low amounts, indicates the existence of a fourth, as yet unidentified bacterial DMSP lyase, but a slow nonenzymatic cleavage of [$^2H_6$]DMSP and DESP cannot be ruled out.

Volatiles from *Dinoroseobacter shibae* DSM 16493^T

The same feeding experiments were carried out with *D. shibae* and lead to similar results to those found for *P. gallaeciensis* and *O. indolifex*, but some important differences were observed (Table S3 and Figure 4). The most striking disparities were the occurrence of large amounts of diethyl sulfone (**11**, from DESP) and small amounts of ethyl methyl sulfone (**10**, from EMSP), but no [$^2H_6$]dimethyl sulfone from [$^2H_6$]DMSP. SPME analysis demonstrated that only traces of [$^2H_6$]DMS close to its detection limit were formed from [$^2H_6$]DMSP (Figure S2). The sulfones **10** and **11** arise from oxidation of ethyl methyl sulfide and **1**, respectively.

The genome of *Rhodobacter capsulatus* 37b4 contains the *dorA* gene, which encodes a dimethyl sulfoxide reductase (DMSOR) that also catalyses the oxidation of DMS to DMSO.[26] A similar DMSOR is encoded in *D. shibae* (Dshi_2278, 35% identity) and might be involved in the oxidation of the dialkyl

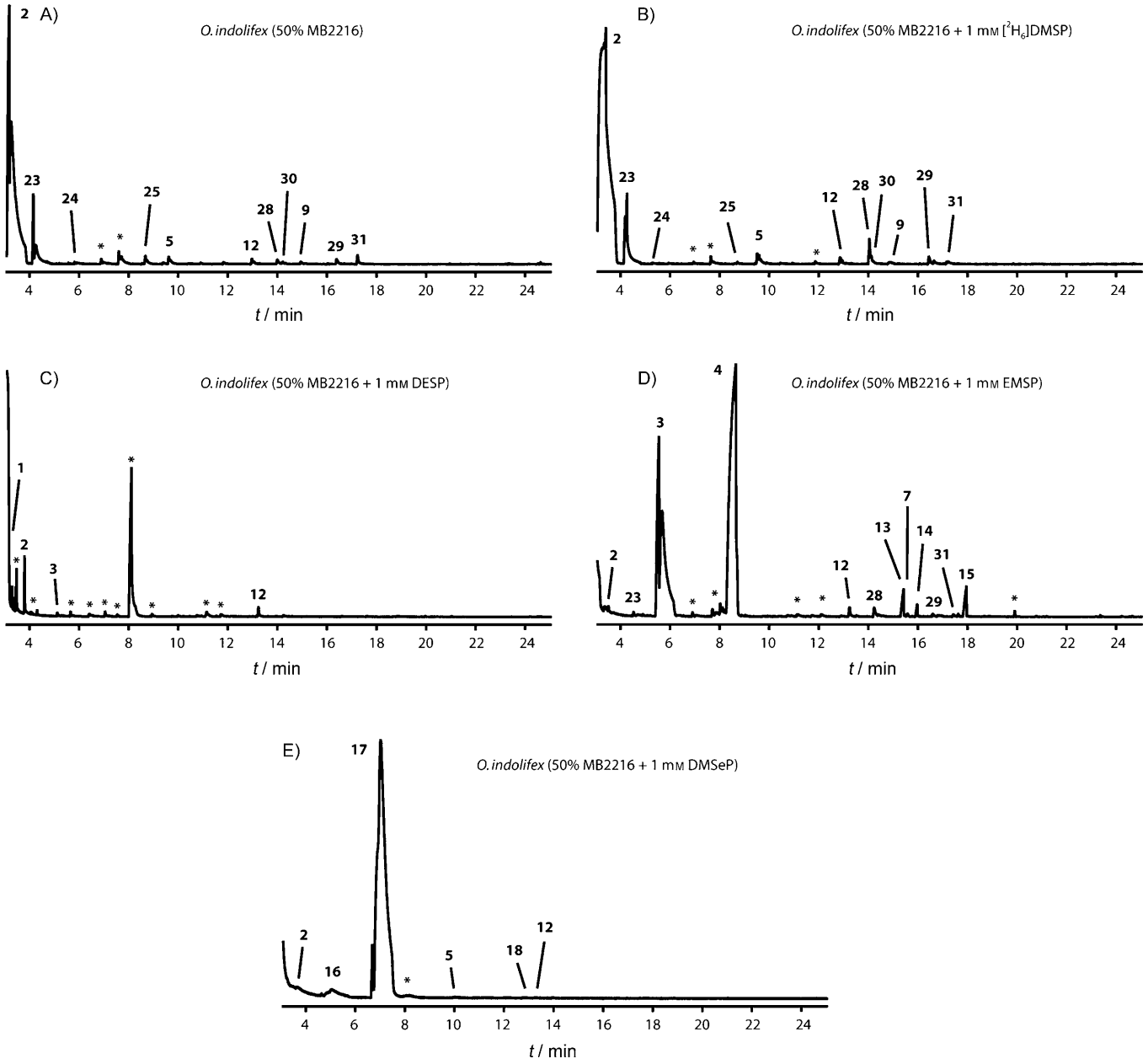


Figure 3. Total ion chromatograms of the CLSA extracts from *O. indolifex* agar plate cultures. The headspace extracts were obtained from A) MB2216 medium, B) MB2216 + 1 mM [$^2H_6$]DMSP·HCl, C) MB2216 + 1 mM DESP·HCl, D) MB2216 + 1 mM EMSP·HCl and E) MB2216 + 1 mM DMSeP·HCl. The peaks are marked with the compound numbers used in the text. Signals arising from the medium, artefacts, and unidentified compounds are labelled with asterisks.

sulfides via the dialkyl sulfoxides to the sulfones. Such an enzyme is not encoded in the genomes of *P. gallaeciensis* and *O. indolifex*, and no dialkyl sulfoxides or sulfones were found in the headspace extracts of these species. The formation of quite high amounts of **11** from DESP in combination with the lack of production of [$^2H_6$]dimethyl sulfone from [$^2H_6$]DMSP in *D. shibae* can be explained by a rapid turnover of DMSP through the dealkylation pathway deviating this substrate from the lysis pathway under the growth conditions used, whereas the blocked dealkylation of DESP results in its lysis and subsequent oxidation to **11**.

EMSP feeding showed that this substrate was efficiently demethylated to yield large amounts of EtSH-derived volatiles. However, after feeding of EMSP, **10** was indeed produced; this points by inversion of the arguments to a blockage of EMSP dealkylation. Although speculative, this apparent contradiction can be solved by assuming a rapid enzyme-catalysed dealkylation of one enantiomer of EMSP, whereas dealkylation of the other enantiomer is blocked to result in its lysis (Scheme 7).

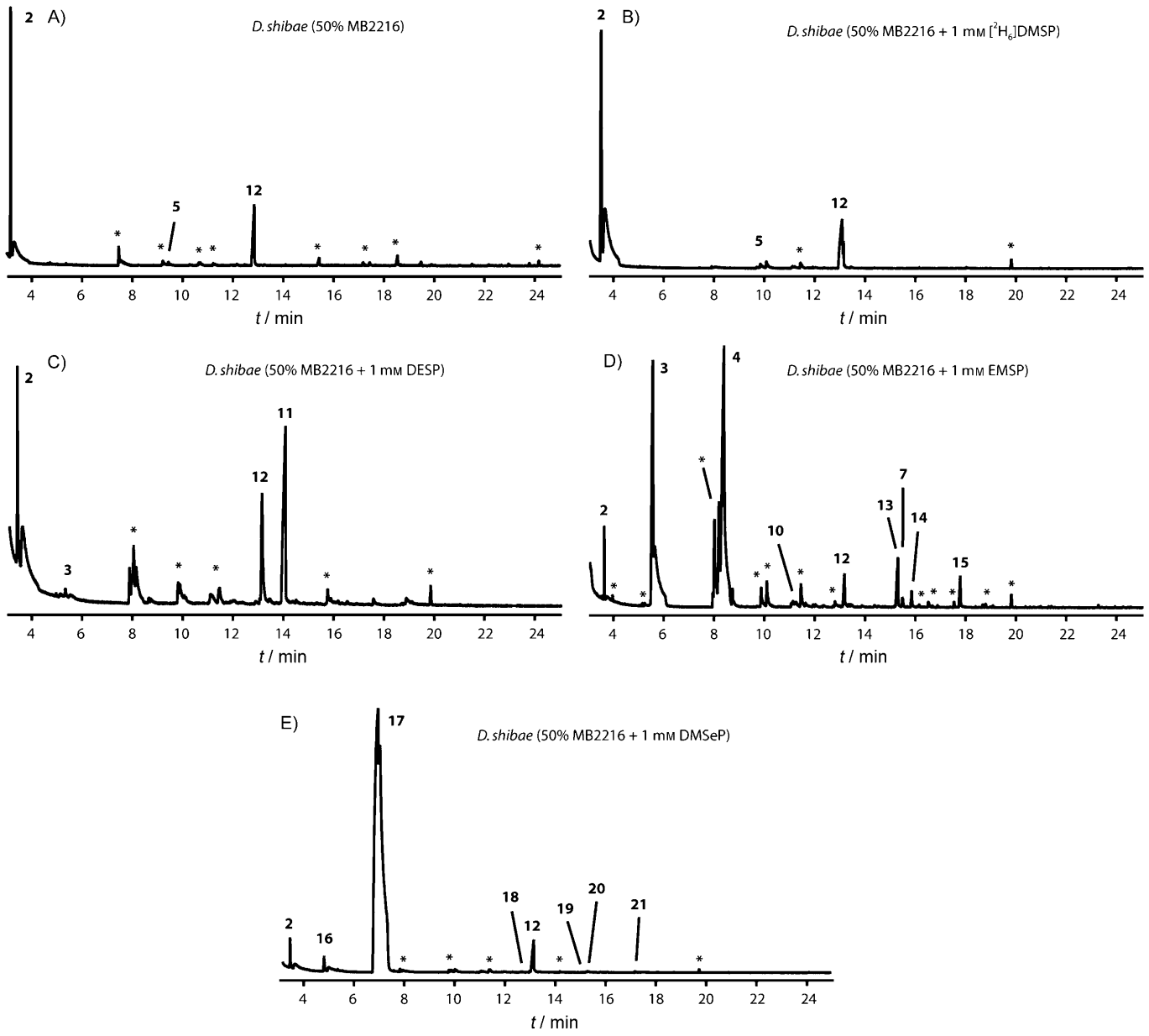
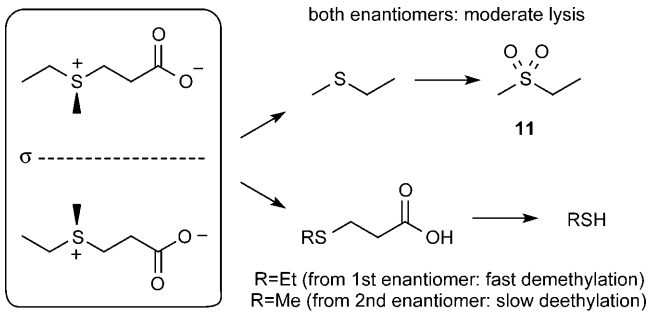


Figure 4. Total ion chromatograms of the CLSA extracts from *D. shibae* agar plate cultures. The headspace extracts were obtained from A) MB2216 medium, B) MB2216 + 1 mM [$^2H_6$]DMSP·HCl, C) MB2216 + 1 mM DESP·HCl, D) MB2216 + 1 mM EMSP·HCl and E) MB2216 + 1 mM DMSeP·HCl. The peaks are marked with the respective compound numbers as used in the text. Signals arising from the medium, artefacts, and unidentified compounds are labelled with asterisks.



Scheme 7. Proposed different turnover rates for the two enantiomers of EMSP via the lysis and the dealkylation pathways.

Occurrence of the identified volatiles in other natural sources

Dimethyl disulfide (**2**) and dimethyl trisulfide (**5**) are widespread bacterial volatiles.[27] The higher homologue dimethyl tetrasulfide (**8**) has been found less frequently, but has been reported from streptomycetes and myxobacteria.[19,28,29] Dimethyl polysulfides also occur in plants, such as the impressively flowering *Amorphophallus titanum*.[30] The EtSH-derived disulfides **3** and **4** are aroma constituents of the durian fruit *Durio zibethinus*, which is distinctive for its unique odour,[31] but have never been reported from bacteria. Sulfonate **12** has previously been detected in other bacteria of the *Roseobacter* clade and from *Stigmatella aurantiaca*,[17,32] and is known from

Allium cepa, which also produces the related compounds *S*-propyl methanethiosulfonate and *S*-propyl propanethiosulfonate.[33] Meanwhile, the EtSH-derived sulfonates **13**–**15** and the sulfones **10** and **11** obtained in our studies from EMSP have never been reported from natural sources.

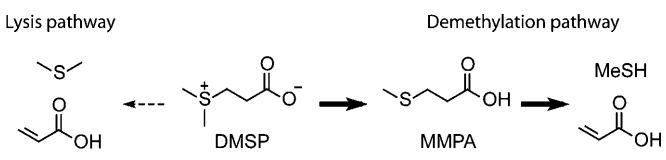
Organoselenium compounds are rare in nature. The bacterial volatiles dimethyl diselenide (**17**) and dimethyl selenyl sulfide (**16**) were previously identified in different *Allium* spp., accompanied by bis(methylthio)selenide, which was not found in our investigations.[34] In addition, **17** and the mixed selenium–sulfur compound **16** are produced by different bacteria supplemented with sodium selenate.[35] Besides **16** and **17**, the trichalcogenide methyl methylseleno disulfide (**18**), but not its isomer bis(methylthio)selenide, is produced from selenate or selenite by genetically modified *Escherichia coli* expressing the UbiE methyltransferase from *Geobacillus stearothermophilus*.[36] The same engineered *E. coli* strain is able to biomethylate potassium tellurate, but not potassium tellurite, to release dimethyl telluride, dimethyl ditelluride and the tellurium analogon of **16**, dimethyl tellurenyl sulfide.[37,38]

The *S*-methyl alkanethioates **23**–**25** are known volatiles from *Streptomyces albidoflavus* and from cheese-ripening bacteria such as *Brevibacterium linens* and *Micrococcus luteus*,[39,40] whereas the selenium analogues **26** and **27** are new natural products. The unusual compound *S*-methyl 3-(methylthio)propanethioate (**31**) is a constituent of human urine and its odour-causing agent after the consumption of asparagus.[41] The structurally related ester **30** is present in different fruits, for example, *Passiflora edulis*.[42] Neither compound has ever been reported from bacteria.

Conclusions

In summary, under the experimental conditions used in this study, DMSP was catabolised by the *Roseobacter* clade bacteria almost exclusively through the demethylation pathway to MeSH, and not through the lysis pathway to DMS (Scheme 8), to result in the release of several MeSH-derived sulfur volatiles, which have all been identified by synthesis. In contrast, DESP catabolism not only yielded sulfur volatiles arising from the dealkylation pathway, but significant amounts of lysis products were found, represented by **1** in *P. gallaeciensis* and *O. indolifex*, whereas the respective oxidation product **11** was emitted by *D. shibae*.

The observed distribution between dealkylation and lysis of DMSP in the *Roseobacter* clade in favour of the dealkylation pathway, in accordance with previous reports,[14] has a high impact on the understanding of the role of bacteria from the *Roseobacter* lineage for the global sulfur cycle and climate, and supports their importance in tightly controlling the sulfur flux from the oceans. The available genetic information alone is not sufficient to predict the used pathways for the bacterial DMSP catabolism, because *P. gallaeciensis* and *D. shibae* both encode enzymes with high homology to known DMSP lyases, whereas the occurrence of **1** in the headspace above *O. indolifex* cultures after feeding of DESP suggests the presence of an unknown type of DMSP lyase in this organism. In addition to a careful genome mining, a chemical analysis of the products of bacterial DMSP catabolism is crucial. While our investigations clearly showed the capacity of agar plate cultures of the *Roseobacter* clade bacteria to produce MeSH-derived sulfur volatiles from DMSP through the dealkylation pathway, the pathway distribution under other (environmental) conditions might be different.



Scheme 8. DMSP catabolism by *P. gallaeciensis*, *O. indolifex*, and *D. shibae* is dominated by the demethylation pathway.

The substrate specificities of the participating enzymes of the DMSP demethylation pathway, that is, the DMSP demethylase(s) and the proposed MMPA lyase, seem to be quite variable. Dealkylation of DMSP derivatives was especially efficient for methylated substrates presented to DmdA (or, alternatively, as yet unknown additional enzymes). Even the sulfur atom could be replaced by selenium, but not tellurium, without significant loss of catalytic activity, whereas the dealkylation of DESP proceeded with significantly reduced activity in all three species. In contrast, lysis of the dealkylation products MMPA, EMPA and MSePA to the alkanethiols or methaneselenol, respectively, was efficient in all cases, and, in other words, was neither dependent on the nature of the chalcogenide-bound alkyl groups nor on the nature of the chalcogenide atom itself. As a consequence of the reduced dealkylation activity towards the unnatural substrate DESP, lysis products were formed by all three species, that were not produced from DMSP. Since the demethylation pathway dominated under the experimental conditions used in our studies, conclusions on the in vivo substrate specificities of the different DMSP lyases encoded in the genomes of the three species under investigation are not possible.

Further experiments on the genetics and enzymology of the DMSP catabolic pathways will be carried out in our laboratories to investigate the substrate specificities of particular enzymes in vitro.

Experimental Section

Strains, culture conditions and feeding experiments: *Phaeobacter gallaeciensis* DSM 17395^T, *Oceanibulbus indolifex* DSM 14862^T and *Dinoroseobacter shibae* DSM 16493^T were precultured in marine broth (MB2216, Roth) at 28 °C with shaking (180 rpm) until an OD_{600} of about 1.0 was reached. For the feeding experiments, MB2216 agar medium (25 mL) was spiked after autoclavation with [$^2H_6$]DMSP·HCl, DESP·HCl, EMSP·HCl, DMSeP·HCl or DMTeP·HCl (1 mM) and poured into glass Petri dishes. (Glass Petri dishes were used to avoid contamination of the headspace extracts with plasticisers.) The agar plates were inoculated with the preculture (100 μL), incubated for two to three days at 28 °C and directly subjected to headspace analysis.

Sampling: The volatile compounds emitted by the bacterial agar plate cultures were collected by using the CLSA headspace technique. Briefly, in a closed apparatus containing the agar plates, a circulating air stream was passed through a charcoal filter (Chrom-

tech GmbH, Idstein, Precision Charcoal Filter, 5 mg) for 24 h. The absorbed volatiles were eluted from the charcoal with analytically pure dichloromethane (30 μL). The obtained solutions were immediately analysed by GC-MS and then stored at −80 °C. For comparison, agar plates containing the medium alone or amended with [$^2H_6$]DMSP·HCl, DESP·HCl, EMSP·HCl, DMSeP·HCl or DMTeP·HCl were investigated in the same way. None of the volatiles discussed in the main text or shown in Tables S1–3 was detected in these experiments. SPME sampling was carried out by drilling a small hole into the (disposable) agar plates and incubating the SPME fibre in the culture headspace for 1 h. Subsequently, the collected volatiles were immediately desorbed from the SPME fibre into the injection port of the GC-MS.

GC-MS: GC-MS analyses were carried out on a HP7890A GC system connected to a HP5975C mass selective detector fitted with a HP-5 fused silica capillary column (30 m × 0.22 mm i.d., 0.25 μm film, Hewlett–Packard). Conditions: inlet pressure: 67 kPa, He 23.3 mL min^{-1}; injection volume: 1 μL; injector: 250 °C; transfer line: 300 °C; electron energy: 70 eV. The GC was programmed as follows: 50 °C (5 min isothermic), increasing at 5 °C min^{-1} to 320 °C and operated in splitless mode (60 s valve time); carrier gas (He): 1.2 mL min^{-1}. Retention indices were determined from a homologous series of *n*-alkanes (C_8–C_{32}). Compounds were identified by comparison of mass spectra to database spectra from the Wiley 7 library, or by comparison to a synthetic reference compound.

Abbreviations

CLSA: closed-loop stripping apparatus, DESP: diethylsulfoniopropionate, DMS: dimethyl sulfide, DMSeP: dimethylseleniopropionate, DMSOR: dimethyl sulfoxide reductase, DMSP: dimethylsulfoniopropionate, DMTeP: dimethyltelluriopropionate, EMPA: 3-(ethylmercapto)propionic acid, EMSP: ethyl methyl sulfoniopropionate, MMPA: 3-(methylmercapto)propionic acid, MSePA: 3-(methylseleno)propionic acid, SPME: solid-phase microextraction.

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Keywords: dimethylsulfoniopropionate · *Roseobacter* · selenium · sulfur · volatiles

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