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Metabolism of DMSP, DMS and DMSO by the cultivable bacterial community associated with the DMSP-producing dinoflagellate *Scrippsiella trochoidea*

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Abstract Bacterial species associated with the dimethylsulfoniopropionate (DMSP)-producing phytoplankton *Scrippsiella trochoidea* were cultured and identified, with the aim of establishing their ability to metabolise DMSP, dimethylsulfide (DMS) and dimethylsulfoxide (DMSO). Results demonstrate that of the cultivable bacteria only α-Proteobacteria were capable of producing DMS from DMSP. The concentration of DMSP was shown to affect the amount of DMS produced. Lower DMSP concentrations (1.5 μmol dm^{-3}) were completely assimilated, whereas higher concentrations (10 μmol dm^{-3}) resulted in increasing amounts of DMS being produced. By contrast to the restricted set of bacteria that metabolised DMSP, ~ 70% of the bacterial isolates were able to 'consume' DMS. However, 98-100% of the DMS removed was accounted for as DMSO. Notably, a number of these bacteria would only oxidise DMS in the presence of glucose, including members of the γ-Proteobacteria and Bacteroidetes. The observations from this study, coupled with published field data, identify DMS oxidation to DMSO as a major transformation pathway for DMS, and we speculate that the fate of DMS and DMSP in the field are tightly coupled to the available carbon produced by phytoplankton.

Keywords Dinoflagellate-associated bacteria · DMSP · DMS and DMSO metabolism · DMS oxidation

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Introduction

Due to its concentration and turnover, dimethylsulfide (DMS) is considered to be one of the most important biogenic sulfur compounds in the marine environment. DMS accounts for over 40% of the total biogenic sulfur entering the atmosphere annually, with about 90% of DMS originating from marine sources (Simó 2001). Evidence for a phytoplankton-cloud-climate feedback loop, in which DMS-derived cloud condensation nuclei affect the Earth's radiation balance by changing the reflectivity of clouds (Charlson et al. 1987), has stimulated considerable research into this gas. Furthermore, and perhaps more significantly, in the marine environment DMS and its precursors represent important sources of carbon, reduced sulfur and energy for bacterioplankton (Kiene and Linn 2000a).

The precursor of DMS, dimethylsulfoniopropionate (DMSP), is produced by marine micro- and macroalgae. Studies examining the distribution of chlorophyll *a* as a measure of phytoplankton biomass from field measurements and satellite observation were hoped to provide a reliable proxy of DMS emissions. However, plots of DMS against chlorophyll *a* resemble scatter-plots, and do not show a sufficiently reliable correlation (Liss et al. 1997). The failure of this measure is due in part to the taxonomic profile of the phytoplankton community; DMSP and DMS concentrations are known to correlate more closely to specific groups of phytoplankton such as the prymnesiophytes and dinoflagellates (Keller et al. 1989). Furthermore, seasonality and the stage of the algal bloom contribute to significant variations in the production of DMS (Ledyard and Dacey 1996; Turner et al. 1988). In the latter case, it has been observed that as the bloom develops, a greater amount of DMS is able to escape biological consumption, as opposed to when the bloom is declining (cell senescence, viral lysis, grazing pressure), where, although there are large quantities of DMS produced, this production is met by a near equal rate of consumption (Archer et al. 2002; van Duyl et al. 1998; Zubkov et al. 2004). Therefore, the DMS concentration in the surface ocean at any given time reflects a complex balance between its biological source, bacterial consumption, photochemical oxidation and ventilation to the atmosphere.

DMSP and its various breakdown products can undergo a number of biological transformations, for which new evidence is continually emerging. The dominant fate for $\sim$75% of dissolved DMSP (Kiene and Linn 2000a) is for it to be assimilated by bacteria, where it can contribute up to 100% of the sulfur and 15% of the carbon requirements of the bacterioplankton community (Simó et al. 2002). The remaining fraction of dissolved DMSP not assimilated, may then undergo cleavage by one or more DMSP lyase-like enzymes (Curson et al. 2008; Todd et al. 2011, 2009; 2007), resulting in the production of DMS. What is not understood is how or why bacteria switch between these two alternative pathways (Rinta-Kanto et al. 2011). This is an important question to answer as it is one of the main regulatory points determining surface ocean DMS concentrations.

In contrast to the appreciable body of literature describing the bacterioplankton community's effect on the fates of DMSP, there has been less research examining the biologically driven fate of DMS in the surface ocean. It has been observed that during incubation experiments DMS was lost from the system, leading to the suggestion that DMS was being consumed as a carbon and sulfur source by bacteria present in surface waters (Zubkov et al. 2002; Vila-Costa et al. 2006), such as by the DMS-utilizing bacteria *Methylophaga* (deZwart et al. 1996). Further evidence has shown that under certain circumstances much of the DMS removed (e.g., 81–93%; del Valle et al. 2007) is not consumed as a source of carbon or sulfur, but instead ends up in a pool of dissolved non-volatile sulfur, primarily comprised of dimethylsulfoxide (DMSO) and sulfate (del Valle et al. 2007; Kiene and Linn 2000b; Vila-Costa et al. 2006; Zubkov et al. 2002). This evidence suggests that DMS oxidation may be a quantitatively important sink for DMS from the surface ocean.

It has been recognised that bacteria can enzymatically oxidise DMS to DMSO (Fuse et al. 1998; Horinouchi et al. 1997; Zeyer et al. 1987), and the work of Vila-Costa et al. (2006) emphasized the likely importance of this process in the field. Recently, we have shown that DMS oxidation may be co-metabolised and could be an important auxiliary energy source for some marine bacteria (Green et al. 2011). Although less is known about the role of DMSO in the marine system, this compound can be present at concentrations similar to or higher than dissolved DMS and DMSP, and may act as a reservoir for DMS. Evidence also suggests that DMSO may be a potential source of DMS following bacterial reduction (as reviewed by Hatton et al. 2004).

Bacterial action on DMSP, DMS and DMSO in the surface ocean does not happen in isolation of the surrounding seawater chemical and biological milieu. Phytoplankton not only produce DMSP, but they also excrete significant quantities of other dissolved organic matter (DOM) into the marine ecosystem. It has been appreciated for several decades that bacteria are actively attracted to phytoplankton because of what they excrete into the space surrounding the algal cell (termed the "phycosphere" or boundary layer; Bell et al. 1974). Indeed, DMSP has been observed to act as a specific chemo-attractant to bacteria (Miller et al. 2004; Zimmer-Faust et al. 1996). Phytoplankton are recognised to contribute significantly to the development of 'microzones' of biochemical stratification in the marine ecosystem (Mitchell et al. 1985), which

rapidly become biodiversity 'hot-spots' (Azam 1998). Thus, the spatial and temporal change to the DOM pool that occurs during the course of an algal bloom—growth, senescence and collapse—is likely to be an important regulatory parameter determining the fate of DMS in the surface ocean. For example, we speculated that the concentration of biologically-available DOM is likely to be an important parameter governing the rate of DMS oxidation by *Flavobacteria* (Green et al. 2011). Furthermore, actively growing, healthy, phytoplankton release relatively small amounts of DMSP into the surrounding water (Turner et al. 1988). Although the dissolved DMSP concentration may be low during bloom development, it has been estimated that the DMSP concentration immediately surrounding the algal cell, may be up to one order of magnitude higher (Scarratt et al. 2000). This is an important observation, firstly, because whatever component of the bacterial community is in close association to the algal cell, it can be assumed that they will have a potentially far greater role in determining the fate of this DMSP/DMS, than those bacteria in the bulk water phase. Secondly, it has been hypothesised that higher concentrations of DMSP will favour DMS production over DMSP/DMS consumption (Kiene et al. 2000).

The aim of this study was to examine the microbial diversity associated with a DMSP-producing dinoflagellate and characterise their metabolic capability with respect to DMSP, DMS and DMSO. The overarching objective was to gain a greater understanding of the interaction of these two kingdoms, in order to yield further insights about the marine DMS biogeochemical cycle, and the importance and potentially close coupling of the fate of DMS to phytoplankton DOM production.

Materials and methods

Bacterial community identification

Bacteria were isolated from a late-log phase *Scrippsiella trochoidea* CCAP 1131/1 culture by serial dilution and growth on ZM/10 agar for 4 weeks, essentially as described by Green et al. (2004). To gain a fuller understanding of the total bacterial diversity of this culture, a culture independent approach was also used. Total bacterial 16S ribosomal RNA genes (16S rRNA) were amplified by PCR and cloned into pGEM-T Easy (Promega). 96 random clones were picked and subjected to DNA sequencing as described in Green et al. (2010). Cultured bacteria were identified by PCR amplification and DNA sequencing of their 16S rRNA. Bacterial 16S rRNA gene sequences were classified according to the RDP II Classifier (Cole et al. 2009). Phylogenetic inference was performed using the ARB software suite (Ludwig et al. 2004) based on the maximum likelihood model. GenBank accession numbers are given in Fig. 1.

Bacterial culture and sole carbon source analysis

Bacterial isolates were maintained on the modified Zobell marine media ZM/10 (except DG1235 which was grown on ZM/1; Green et al. 2004). Cultivable bacteria were tested for their growth on glucose, DMSP, DMSO and acrylic acid as sole carbon sources. Cells were harvested from agar plates and grown to late exponential phase in liquid ZM/10 medium in dark at $\sim$21°C at $\sim$100 rpm. Cells were then washed twice by centrifugation (8,000×*g* for 15 min) and suspended in equal volumes of basal medium (Tris–HCl was omitted because DG1231 was able to utilise Tris–HCl as a sole carbon source) containing 0.1 mmol dm^{-3} Fe-EDTA and vitamins (González et al. 1999). Washed cells were added to triplicate wells of a 96-well microplate to which DMSP, DMSO, acrylic acid or glucose were added at a final concentration of 1 mmol dm^{-3}. Growth was measured spectrophotometrically (A_{540nm}) daily up to 10 days using a BioTek microplate reader (EL-340). Growth was scored as positive (+; $P < 0.01$) or weak (w; $P < 0.05$) where maximal absorbance of triplicate wells was significantly greater than no-carbon containing controls (Student's *t*-test).

Metabolism of DMSP, DMS and DMSO

Initial survey of cultivable bacterial isolates

To establish which of the cultivable bacteria metabolised DMSP, DMS or DMSO, each isolate was grown in ZM/10 at $\sim$21°C in the dark with orbital shaking (100 rpm) to stationary phase. Following growth, the cells were washed twice as described above, except that the basal medium contained Tris–HCl as per originally described (González et al. 1999). The cells were finally suspended in 5 volumes of basal medium and 1 ml was added per 10 ml crimp-top glass vial containing final

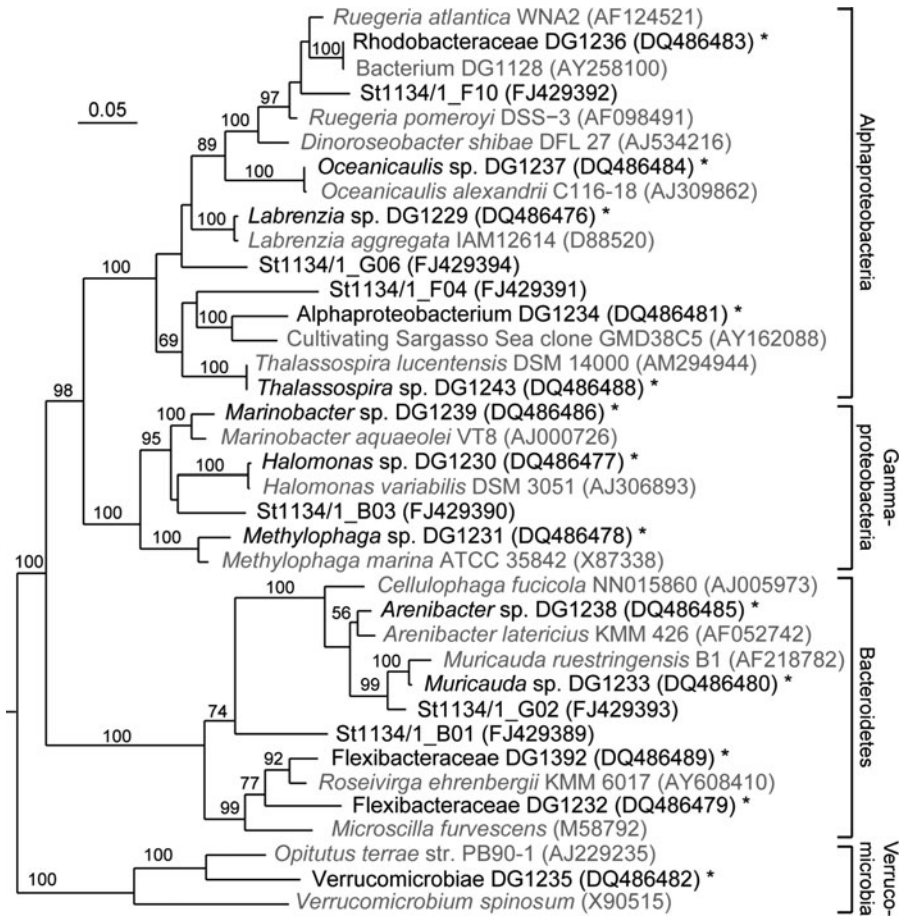

Fig. 1 Phylogenetic analysis of the *S. trochoidea* bacterial community. Maximum likelihood 16S rRNA gene dendrogram of cultivable strains analysed in this study (*asterisk*) and cloned bacteria isolated from *S. trochoidea* CCAP 1134/1 (*black text*) and publically available reference sequences (*grey text*). GenBank accession codes are shown in *brackets*

concentrations of DMSP (10 μmol dm^{-3}) or DMS (1.5 μmol dm^{-3}) with or without glucose (5 mmol dm^{-3}). Vials were sealed with PTFE-lined crimp tops. Control vials were set up to check for chemical degradation of substrates (without bacteria) and for any production of biogenic sulfur compounds produced by bacteria alone (vials with bacteria and glucose only). All vials were incubated in the dark (~21°C) to minimise any photochemical oxidation of DMS. Duplicate vials were sacrificed at 12 and 24 h and analysed for DMSP, DMS and DMSO as described below.

Detailed metabolism experiments on three model species

Following initial results, detailed experiments were conducted on strains shown to exhibit DMSP metabolism (DG1229 and DG1236) and DMS oxidation (DG1233). For DG1229 and DG1236, cells were grown in ZM/10 broth and washed following the procedure described above. A final volume of 1 ml of washed cell suspension was added per 10 ml glass crimp top vial, each containing a final concentration of either 1.5 μmol dm^{-3} or 10 μmol dm^{-3} DMSP, with or without glucose (5 mmol dm^{-3}), and sealed as above. Controls were as described above. Samples were incubated in the dark at ~21°C. Triplicate vials were sacrificed at 0, 12, 24, 48 and 72 h and analysed for DMSP, DMS and DMSO. 0.2 ml of each sample was removed and fixed with 0.5% glutaraldehyde and stored at 4°C prior to flow cytometry. For DG1233, cells were grown and washed as above. Washed cells were added to 10 ml crimp top vials containing a final concentration of either 1.5 μmol dm^{-3} or 10 μmol dm^{-3} DMS, with and without glucose (5 mmol dm^{-3}). Controls were as described above. Triplicate vials were sacrificed for measurement at 0, 6, 12, 24, 48 and 72 h and analysed for DMS and DMSO and 0.2 ml of each sample were fixed with glutaraldehyde (0.5%) and stored at 4°C prior to flow cytometry.

The fixed bacterial samples were stained with SYBR Green I (Invitrogen) and were enumerated by flow cytometric analysis on a FACSort instrument (Becton–Dickinson) as described (Zubkov et al. 2001b).

Analysis of biogenic sulfur compounds

For each sample, DMS was purged directly from the vial and cryo-trapped prior to immediate analysis using a Varian 3400 gas chromatograph (GC) fitted with a dual flame photometric detector (FPD). Following DMS measurement, aliquots were taken for DMSP and DMSO analysis. DMSO samples were analysed immediately where possible following its breakdown to DMS using the DMSO reductase method (Hatton et al. 1994). If immediate analysis was not possible, samples were stored frozen (−20°C) for a maximum of 2 weeks prior to analysis. DMSP was measured as DMS following its hydrolysis using 10 mol dm^{-3} sodium hydroxide as detailed in Turner et al. (1990). DMSP (Research Plus, NJ) and DMSO (Sigma) were used as standards. Standards were prepared and analysed under experimental conditions as per standard practice. As such standards for DMS analysis were prepared in crimp top bottles and purged directly. Standards were also prepared containing DOC at the concentrations used in experiments to ensure no interference with analysis. The detection limit for DMS, DMSP and DMSO was 10 nmol dm^{-3}, with a coefficient of variation of less than 4% for repeat runs of standards and samples.

Results

Bacterial community diversity and growth characteristics

Cultivation and cultivation-independent methods (16S rRNA gene clone library and DNA sequencing) were used to identify the bacterial community associated with *S. trochoidea* CCAP 1134/1. A total of 19 bacterial phylotypes were identified, of which 13 were cultivable (Fig. 1). α-Proteobacteria comprised the largest percentage (42%) of the identified diversity, while Bacteroidetes (32%), γ-Proteobacteria (21%) and Verrucomicrobia (5%) made up the balance of total community diversity. The ability of each of the cultivable bacterial strain to grow on a range of sole carbon sources was determined following growth in a basal salt medium containing nitrogen, phosphorus, trace elements and vitamins (González et al. 1999). Results demonstrated that only two of the 13 bacteria examined were capable of growth on DMSP (Table 1). Both belong to the α-Proteobacteria. It was observed that two strains of γ-Proteobacteria and

Table 1 Growth of *S. trochoidea*-associated bacteria on different sole carbon sources

Strain	Glucose	DMSP	DMSO	Acrylate
α-Proteobacteria				
DG1229	+	w	w	+
DG1234	−	−	−	−
DG1236	+	w	−	−
DG1237	−	−	−	−
DG1243	+	−	−	−
γ-Proteobacteria				
DG1230	+	−	−	w
DG1231	−	−	−	−
DG1239	+	−	−	+
Bacteroidetes (∼CFB)				
DG1232	+	−	−	−
DG1233	+	−	−	−
DG1238	+	−	−	−
DG1392	−	−	−	−
Verrucomicrobia				
DG1235	+	−	−	−

+ Positive growth; *w* weak growth; − no growth

Growth was considered positive ($P < 0.01$) or weak ($P < 0.05$) where maximal absorbance of triplicate wells was significantly greater (Student's *t*-test) than no-carbon containing controls

one of the α-Proteobacteria were able to utilise acrylate, which is a product of DMSP catabolism. Finally, only one strain, DG1229 from the α-Proteobacteria, was shown to utilise DMSO as a sole carbon source under the conditions tested.

Metabolism of DMSP by cultivable bacterial strains

Initial survey of DMSP metabolism on cultivable bacterial isolates

Each of the cultivable bacterial strains were analysed for their ability to metabolise DMSP. Results (Table 2) show that utilisation of DMSP, either by demethylation or dissimilation to DMS, was restricted to the two members of the α-Proteobacteria shown to utilise DMSP as sole carbon source (DG1229 and DG1236; Table 1). Results indicated that for both strains, incubated with 10 μmol dm^{-3} DMSP, between ~76 and 91% of the DMSP was assimilated within 24 h. The remainder was dissimilated to DMS, and ultimately converted to DMSO, accounting for between 9 and 24% of the total DMSP added (Table 2). Removal of DMSO was not observed in these experiments.

Detailed DMSP metabolism experiments using model species

To fully assess metabolism of DMSP by both DG1236 and DG1229 a more detailed time series experiment was conducted using two different DMSP concentrations (1.5 and 10 μmol dm^{-3}). For the *Roseobacter*-related bacterium DG1236 results clearly demonstrated that at 10 μmol dm^{-3} DMSP was rapidly metabolised with or without glucose present, resulting in >80% removed within the first 12 h, and the concentration below the detection limit (<10 nmol dm^{-3}) within 48 h (Fig. 2). Results indicated that there was little difference between the rate of removal with and without glucose, with 0.68 μmol dm^{-3} h^{-1} lost over the first 12 h in the presence of glucose, and 0.70 μmol dm^{-3} h^{-1} lost over the same time period without. Loss of DMSP coincided with an increase in the concentration of DMS in the first 12 h of the incubations with concentrations up to 1.6 μmol dm^{-3} recorded with glucose, and 1.43 μmol dm^{-3} without, accounting for ~15% of the initial 10 μmol dm^{-3} DMSP added dissimilated to DMS. After 12 h the concentration of DMS declined slightly, with a concomitant increase in the concentration of DMSO, accounting for all the DMS lost (Fig. 2). Following an initial lag period, bacterial numbers increased over the first 48 h in both treatments, with

Table 2 DMSP metabolism by cultivable bacterial strains from *S. trochoidea*. Experiments were carried out at 10 μmol dm^{-3} DMSP, with (G) and without (NG) glucose

Strain	% of DMSP dissimilated[a]		% of DMSP assimilated[b]	
	G	NG	G	NG
α-Proteobacteria				
DG1229[#]	9	21	91	79
DG1234	–	–	–	–
DG1236[#]	24	19	76	81
DG1237	–	–	–	–
DG1243	–	–	–	–
γ-Proteobacteria				
DG1230	–	–	–	–
DG1231	–	–	–	–
DG1239	–	–	–	–
Bacteroidetes (~CFB)				
DG1232	–	–	–	–
DG1233[c]	–	–	–	–
DG1238	–	–	–	–
DG1392	–	–	–	–
Verrucomicrobia				
DG1235	–	–	–	–

– No loss of DMSP recorded over 24 h

[a] % DMSP dissimilation calculated from total DMS + DMSO at 24 h

[b] In both cases[#] DMSP concentrations were below detection limit by the end of the experiment showing that the remaining DMSP was assimilated

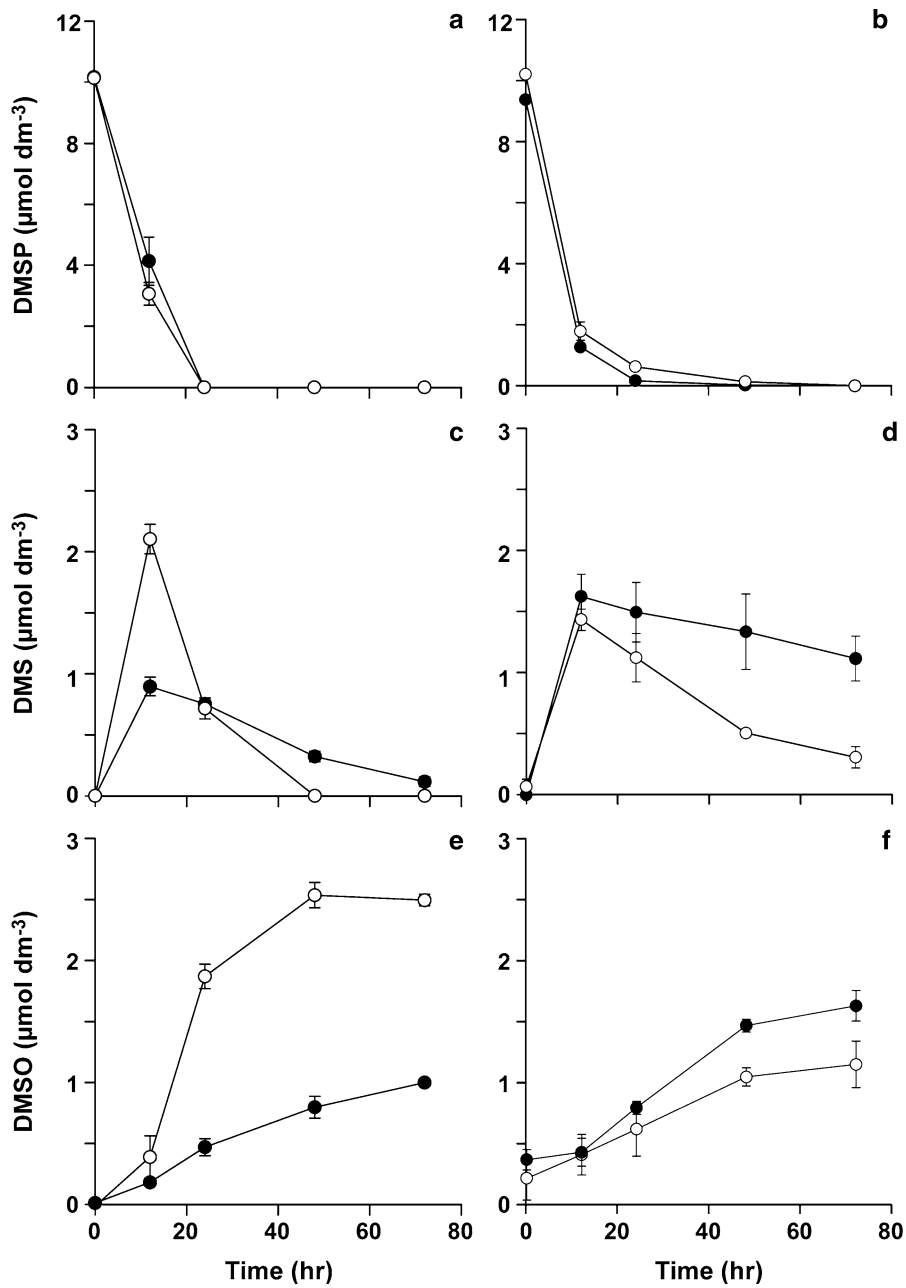

Fig. 2 Time course experiment for DMSP metabolism. DG1229 (**a**, **c**, **e**) and DG1236 (**b**, **d**, **f**) were incubated with 10 µmol dm^{-3} DMSP. *Filled circle*, glucose added; *open circle*, no glucose added. *Error bars* represent SD of the mean of triplicate samples

maximum cell numbers of 4.2×10^7 cells ml^{-1} in the presence of glucose and 4.0×10^7 cells ml^{-1} when glucose was absent.

Experiments were repeated with 1.5 µmol dm^{-3} DMSP to assess if DMSP concentration influenced the metabolic fate and the rate of removal. Results for DG1236 show again that DMSP was rapidly metabolised (Fig. 3), with or without glucose present. However, at this concentration the presence of glucose appears to have a more pronounced effect, with all DMSP removed after 24 h at a rate of 0.08 µmol dm^{-3} h^{-1} in the presence of glucose, compared to ~68% of the DMSP removed within the first 24 h at a rate of 0.066 µmol dm^{-3} h^{-1} when glucose was absent. Notably, no increase in the DMS concentration was observed in either treatment, but a small increase

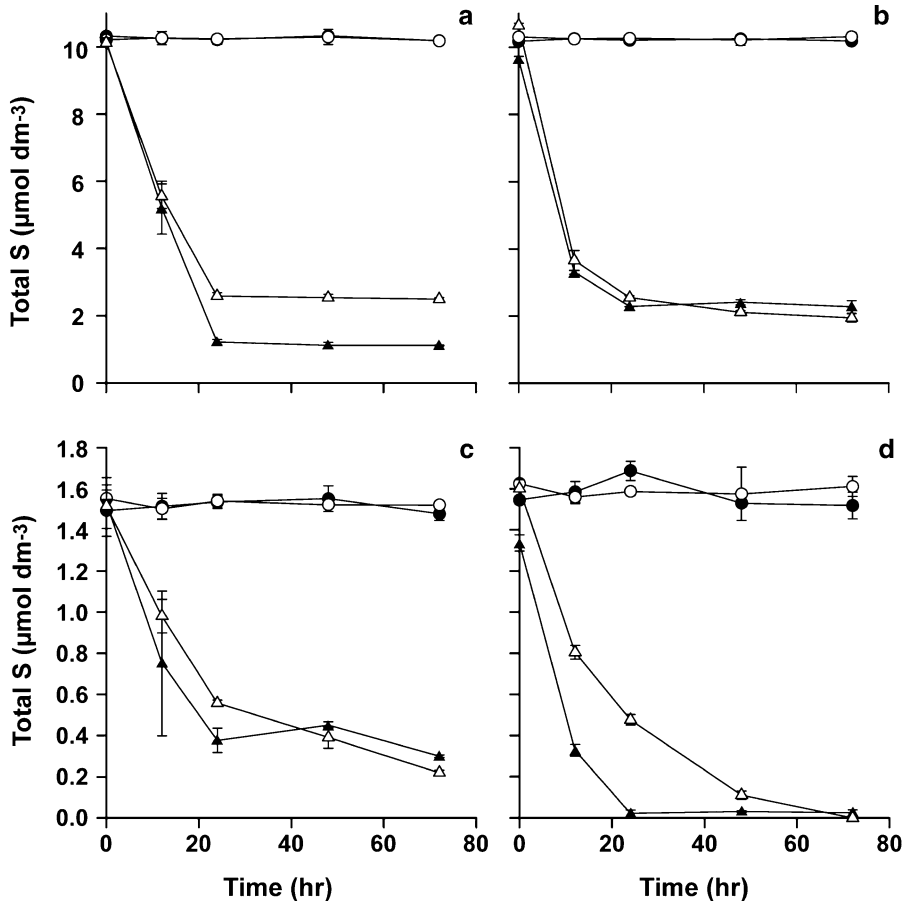

Fig. 3 Total sulfur concentrations recorded during time course experiments for DMSP metabolism in strains DG1229 (*Triangles*; **a**, **c**) and DG1236 (*Triangles*; **b**, **d**). DMSP was added at a final concentration of 10 (**a**, **b**) and 1.5 (**c**, **d**) µmol dm^{-3}, with glucose (*filled symbols*) and without glucose (*open symbols*). Control vials (*circles*) were set up to check for chemical degradation of substrates (without bacteria). Total sulfur represents the combined concentrations of DMSP + DMS + DMSO, giving an indication of the total amount of the analysed sulfur compounds lost through assimilatory versus dissimilatory pathways. *Error bars* represent SD of the mean of triplicate samples

in the DMSO concentration was observed (~0.03 µmol dm^{-3} DMSO) in the presence of glucose only. Flow cytometry data indicated that cell numbers increased to 2.0×10^7 cells ml^{-1} without glucose and 2.5×10^7 cells ml^{-1} in the presence of glucose during the first 48 h, with no further increase observed.

For *Labrenzia* sp. DG1229, results also demonstrated that at 10 µmol dm^{-3} DMSP was rapidly metabolised, with or without glucose present, with between 60 and 70% removed within the first 12 h and concentration below detection limit within 24 h (Fig. 2). Results showed that there was a small difference between the rate of removal with and without glucose, with 0.50 µmol dm^{-3} h^{-1} lost over the first 12 h in the presence of glucose and 0.59 µmol dm^{-3} h^{-1} lost over the same time period without glucose. Loss of DMSP coincided with an increase in the concentration of DMS in the first 12 h of the incubations with concentrations up to 0.89 µmol dm^{-3} recorded with glucose and 2.1 µmol dm^{-3} without (Fig. 2). In contrast to DG1236, a marked difference in the total amount of DMSP dissimilated in the presence or absence of glucose was observed, accounting for ~9 and ~21%, respectively of the 10 µmol dm^{-3} added. After 12 hours the concentration of DMS declined again with a concomitant increase in the concentration of DMSO. Flow cytometry data indicated that in the presence of glucose cell numbers experienced an initial lag of 24 h then increase rapidly to a maximum of

4.0×10^7 cells ml^{-1} at 72 h. However, in the absence of glucose no increase in cell numbers was observed.

Experiments with DG1229 were also repeated at 1.5 μmol dm^{-3} DMSP. Results showed again that DMSP was metabolised, with or without glucose (Fig. 3). However, DMSP was metabolised at a slower rate, with ~0.2 μmol dm^{-3} DMSP remaining after 72 h. At this concentration the presence of glucose appears to have a small effect on DMSP removal rate in the first 24 h, with DMSP removed at a rate of 0.07 μmol dm^{-3} h^{-1} in the presence of glucose but at 0.05 μmol dm^{-3} h^{-1} when glucose was absent. No increase in the concentration of DMS was observed in the absence of glucose, but a small increase in DMS was observed (~0.02 μmol dm^{-3}) when glucose was present. Both treatments showed some DMSO production reaching a maximum concentration for both after 48 h with 0.06 and 0.05 μmol dm^{-3} DMSO observed. Flow cytometry data indicated that in the presence of glucose cell numbers experienced an initial lag of 24 h then increase rapidly to a maximum of 3.1×10^7 cells ml^{-1} at 72 h. Again no increase in cell numbers was observed in the absence of glucose.

Metabolism of DMS and DMSO by cultivable bacterial strains

Initial survey of DMS and DMSO metabolism on cultivatable bacterial isolates

None of the bacterial strains isolated from *S. trochoidea* appeared to be capable of assimilating DMS or DMSO as a carbon/sulfur source under the conditions tested in our incubation experiments. Whilst *Methylophaga* sp. DG1231 did not utilise any of the carbon sources provided (Table 1) or DMS (Table 3), it did grow on methanol (data not shown). None of the bacterial species tested assimilated DMS, however, 9 of the 13 bacterial species tested, including the 2 α-Proteobacteria shown to metabolise DMSP, did

Table 3 Percentage DMS oxidised and DMS oxidation rates by the cultivable bacterial strains from *S. trochoidea*, in the presence of 1.5 μmol dm^{-3} DMS with (G) and without (NG) glucose

Strain	% DMS oxidised[b]		DMS oxidation[a] (nmol dm^{-3} h^{-1})	
	G	NG	G	NG
α-Proteobacteria				
DG1229	71	100	17.7	53.1
DG1234	0	0	–	–
DG1236	44	42	17.9	11.2
DG1237	0	8	–	1
DG1243	69	20	20.7	5.7
γ-Proteobacteria				
DG1230	55	0	32.5	–
DG1231	0	0	–	–
DG1239	0	0	–	–
Bacteroidetes (~CFB)				
DG1232	46	0	12.8	–
DG1233[c]	98	0	30.0	–
DG1238	97	0	13.1	–
DG1392	86	0	26.4	–
Verrucomicrobia				
DG1235	100	100	37.3	36.7

– No detectable oxidation or assimilation of DMS was detected

[a] Rates were calculated based on DMSO formed over the first 24 h period

[b] Percentage of initial DMS lost over the 24 h incubation

[c] DG1233 was also tested at 10 μmol dm^{-3} DMS, and the oxidation rate was calculated to be ~122 nmol dm^{-3} h^{-1} when glucose was present

remove DMS (Table 3). However, in each case the full amount of the DMS lost could be accounted for via the formation of DMSO (Table 3). DMS oxidation rates were calculated for each species, both in the presence and absence of glucose. Results showed oxidation rates of between 9.1 and 37.3 nmol dm^{-3} h^{-1} with glucose, but without glucose a more restricted set of bacteria were capable of DMS oxidation, with rates ranging between 1 and 53.1 nmol dm^{-3} h^{-1} (Table 3). At least one member from each bacterial phylogenetic group demonstrated an ability to oxidise DMS to DMSO. Notably, DMS oxidation by α-Proteobacteria and Verrucomicrobia appeared to be independent of a requirement for glucose (Table 3). Whereas bacterial strains from the Bacteriodetes and γ-Proteobacteria all required an additional carbon source (glucose) before DMS oxidation occurred.

Detailed DMS metabolism experiments on Muricauda sp. DG1233

In order to investigate the fate of DMS in greater detail, the Bacteroidetes strain DG1233 was selected as a model species and DMS oxidation was followed through time. Results showed that for 1.5 μmol dm^{-3} DMS addition experiments, DMS was rapidly oxidised with 92% removed within 48 h and 100% lost within 72 h when glucose was present (Fig. 4). On average, >99% of the DMS removed could be quantitatively accounted for as DMSO. The DMS concentration did not appear to affect the fate of DMS, as all of it was removed and accounted for as DMSO within 72 h at both 1.5 and 10 μmol dm^{-3} DMS concentrations. DMS oxidation rates were calculated to be ~30 and 122 nmol dm^{-3} h^{-1} for 1.5 and 10 μmol dm^{-3} DMS respectively (Table 3). Results also confirmed our initial findings that no significant oxidation of DMS occurred in the absence of glucose (Fig. 4). Cell counts showed that in the presence of glucose cell numbers showed an initial lag phase followed by a rapid increase in cell numbers after 24 h with maximum cell numbers of 1.3×10^7 cells ml^{-1} at 72 h. No significant increase in cell numbers was observed without glucose present. Results therefore support the initial sole carbon source experiment showing that DG1233 is capable of growth on glucose as a sole carbon source, but not DMS.

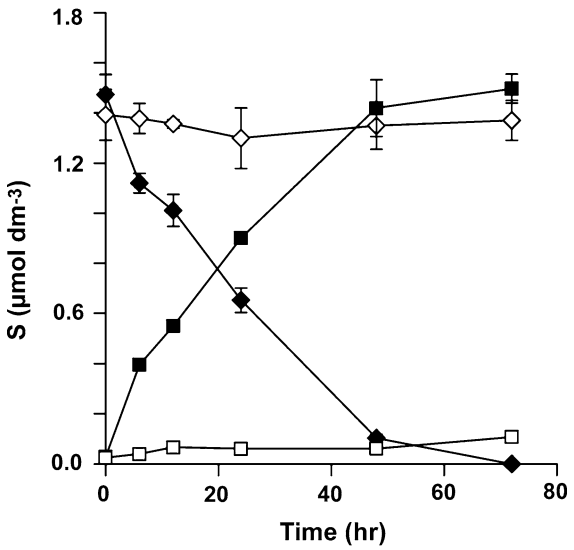

Fig. 4 Time course experiment for DMS metabolism. *Muricauda* sp. DG1233 incubated with 1.5 μmol dm^{-3}, with glucose (*filled symbol*) and without glucose (*open symbol*). *Diamonds* DMS (μmol dm^{-3}); *Squares* DMSO (μmol dm^{-3}). *Error bars* represent SD of the mean of triplicate samples

Discussion

A total of 19 bacterial phylotypes were identified associated with the marine dinoflagellate *S. trochoidea,* belonging to a diversity of bacterial phyla including α-Proteobacteria, Bacteroidetes, γ-Proteobacteria and Verrucomicrobia. These bacterial groups, and in particular the dominance of α-Proteobacteria and Bacteroidetes, are taxonomically representative of the typical bacteria found in algal blooms in the field (e.g., Fandino et al. 2001, 2006; Zubkov et al. 2001a; DeLong et al. 1993). Thus, our view is that the cultivable bacteria isolated from this dinoflagellate will likely represent all or most of the major metabolic pathways affecting the fate of DMSP in the productive surface ocean. Furthermore, as *S. trochoidea* produces abundant quantities of DMSP (Hatton and Wilson 2007), the bacterial community associated with this dinoflagellate culture is expected to be well adapted to the turnover of DMSP and its breakdown products.

Sole carbon source experiments demonstrated that of the 13 cultivable bacteria only two, DG1229 and DG1236, both belonging to the α-Proteobacteria, were capable of growth on DMSP. This is consistent with literature reporting members of the α-Proteobacteria as important and abundant members of the marine

microbiota capable of utilising DMSP (González et al. 1999; Zubkov et al. 2001a). It was also observed that two strains of γ-Proteobacteria and one of the α-Proteobacteria were able to utilise acrylate. So while γ-Proteobacteria could not utilise DMSP directly, they show an adaptation to the utilisation of a breakdown product of DMSP metabolism, suggesting that syntrophic interactions occur amongst the bacteria associated with DMSP-producing dinoflagellates. Finally, only DG1229 was shown to be able to utilise DMSO as a sole carbon source, with weak growth detected after 10 days incubation with 1 mmol dm^{-3} DMSO. However, it should be noted that in our detailed incubation experiments conducted at lower concentrations, no measurable removal of DMSO by DG1229 was observed.

Metabolic pathway experiments confirmed that of the thirteen cultivatable bacterial strains tested only two, DG1236 and DG1229, could metabolise DMSP. Both strains appeared to be capable of metabolising DMSP via both DMSP assimilatory pathways (e.g., demethylation) and DMSP dissimilatory pathways leading to the formation of DMS (e.g., DMSP lyase). Furthermore, although neither strain appeared to be capable of assimilating DMS as a source of carbon or sulfur, both demonstrated the ability to oxidise DMS to DMSO. We had expected that DG1236 would metabolise DMSP as it is closely related to members of the *Roseobacter* clade, most of which readily utilise DMSP (González et al. 1999), via either the demethylation pathway (Howard et al. 2006) or lysis pathways (Curson et al. 2008; Todd et al. 2011, 2009, 2007). However, a number of studies have suggested that DMSP assimilation can be attributed to a wider range of marine bacterioplankton (Howard et al. 2006, 2008; Malmstrom et al. 2004; Tripp et al. 2008; Vila-Costa et al. 2010), and so we had expected that a number of the other associated bacterial strains would metabolise DMSP. It may be that some of the non-cultivable α- and γ-proteobacterial phylotypes (Fig. 1) were also capable of DMSP assimilation. The absence of any detectable DMS assimilation was unexpected as *Methylophaga* sp. DG1231 and *Labrenzia* sp. DG1229 are both closely related to bacteria that utilise C_1 carbon sources, namely DMS and carbon monoxide, respectively (deZwart et al. 1996; Schäfer 2007; Weber and King 2007). However, DG1231 did demonstrate it was methylotrophic, but that its preference was for methanol rather than DMS.

For both DG1229 and DG1236, DMSP concentration appeared to affect both the rate of DMSP metabolism and the percentage of DMSP metabolised via the assimilation and dissimilation pathways (Fig. 3). At 10 μmol dm^{-3} DMSP was removed at an average rate of 0.61 ± 0.09 μmol dm^{-3} h^{-1} compared to 0.066 ± 0.012 μmol dm^{-3} h^{-1} for 1.5 μmol dm^{-3} DMSP. This equated to an increase in the rate of removal of ~0.153 μmol dm^{-3} h^{-1} for every additional μmol dm^{-3} of DMSP added. At the higher DMSP concentration, between ~75 and 91% of the DMSP removed was assimilated by the cells. The remainder was converted to DMS, which was then oxidised to DMSO, overall accounting for ~9–25% of the total DMSP added. However, at 1.5 μmol dm^{-3} between 96 and 100% of the DMSP was assimilated, with little or no DMS or DMSO detected (Fig. 3). This implies that when DG1236 or DG1229 encounter concentrations of DMSP in excess of cellular demands, they will cleave DMSP to DMS, which generates an energy and carbon flow to the cell (de Souza and Yoch 1995). Moreover, the clear implication from our data is that these bacteria can then rapidly use this DMS to generate a further energy flow through DMS oxidation (Zeyer et al. 1987). However, at lower DMSP concentrations (≤1.5 μmol dm^{-3}) most, if not all of the DMSP will be assimilated. This evidence and the sole carbon source results (Table 1) indicate that both strains are primarily using DMSP as a carbon and energy source, and producing DMS when there is sufficient C and or S supply.

Ecologically, 10 μmol dm^{-3} DMSP is a high concentration for the sea surface. However, as the concentration of DMSP within phytoplankton such as *S. trochoidea* can be in the millimolar range (Hatton and Wilson 2007), the rapidity with which both DG1229 and DG1236 metabolise DMSP implies an adaptation of their enzyme systems to higher concentrations of DMSP. This reinforces the hypothesis that algal-associated bacteria may be ecologically important to the rapid turnover of a large quantity of the available DMSP, despite their absolute abundance in the field being comparatively small. Work by Scarratt et al. (2000) showed that from fractionated field water samples the rate of DMS production was ~10-fold higher for algal-attached bacteria than free-living bacteria. Their explanation for this disparity was that attached bacteria were used to experiencing higher concentrations of DMSP than free-living bacteria.

Overall, the observations of Scarratt et al. (2000) and those of this study, suggest that algal- and particle-associated bacteria may be particularly important to DMSP turnover.

α-Proteobacteria DG1236 and DG1229 were observed to oxidise DMS to DMSO, but this was not a phenomenon unique to the DMSP-catabolising bacteria. An additional seven bacterial strains associated with *S. trochoidea* were also shown to oxidise DMS to DMSO (Table 3). Thus, DMS oxidation was observed in strains from all the phyla identified (Proteobacteria, Bacteroidetes and Verrucomicrobia), indicating that this phenomenon is common to a wide diversity of algal-associated bacteria, and the logical likelihood is that this includes a wide range of the surface ocean bacterioplankton exposed to DMS. However, an important separation in the way these bacteria oxidised DMS was observed. First, DMS oxidation by the α-Proteobacteria and Verrucomicrobia strains was independent of any exogenous carbon source (glucose) being present—although glucose availability did change the rates of oxidation of some strains (Table 3). Second, DMS oxidation by the γ-Proteobacteria and Bacteroidetes strains was absolutely contingent on an exogenous carbon source being available. The significance of the two modes of DMS oxidation is several-fold. First, it indicates that the enzyme(s) or pathways involved in DMS oxidation in the α-Proteobactera (and Verrucomicrobia) are potentially different to that in the Bacteroidetes and γ-Proteobacteria. Second, the requirement of glucose for DMS oxidation by the latter phyla suggests this is a co-metabolic process, where a "growth substrate", glucose, is required for the oxidation of the "non-growth substrate", DMS (Dalton et al. 1982). If this is co-metabolism, then it is likely that any available "growth substrate" will suffice for DMS oxidation. This has significant implications to the field situation, for example during a bloom crash, where there is an abundant supply of fresh DOM but also a lot of DMS being produced. The net result could be that while DMS production rates are high, there could be equally rapid rates of DMS removal via its oxidation to DMSO.

Much of the literature reporting DMS removal from the sea surface has used the term, 'bacterial consumption'. In most of these cases, the studies monitored the loss of DMS as an indicator of this pathway, but did not measure DMSO. Our results and those recently published by others using radioactive tracer techniques (del Valle et al. 2007; Vila-Costa et al. 2006; Zubkov et al. 2002) indicate that bacterial assimilation of DMS as a carbon source is a relatively minor pathway, representing only ~2% of the DMS consumed by natural bacterial populations in the Sargasso Sea (del Valle et al. 2007), North Sea (Zubkov et al. 2002) and Gulf of Mexico (Vila-Costa et al. 2006). Instead, there is now a body of evidence, which supports the hypothesis that DMS oxidation to DMSO may be the major pathway by which DMS is removed from marine surface waters (del Valle et al. 2007, 2009). Previous studies have also indicated that glucose affects the percentage of DMS oxidised to DMSO. Vila-Costa et al. (2006) showed that in the presence of glucose or in fresh samples containing natural DOC, 88 and 70%, respectively, of the DMS consumed was lost through oxidation to DMSO. By contrast, amendment of DMS to treatments depleted of carbon resulted in only ~10% of the DMS being removed to DMSO. Instead, 88% was transformed to sulfate and the remainder assimilated into macromolecules. The clear implication from this and other work is that an available carbon source is a key parameter determining the rate of DMS removal to DMSO.

The availability of an alternative carbon source, such as glucose, not only had implications for the oxidation of DMS by members of the Bacteroidetes and γ-Proteobacteria, but also influenced the amount of DMS produced in the first instance. Results for the two α-Proteobacteria show that although both appear to be capable of modest growth on DMSP, both showed better growth when utilizing glucose as a sole carbon source. Thus, growth rate and associated cellular carbon and sulfur demand are regulating the fate of DMSP (Kiene et al. 2000). For DG1236 (*Roseobacter*-related), enhanced growth in the presence of glucose resulted in an increased amount of DMSP dissimilated to DMS and DMSO rather than assimilated through the demethylation pathway. For DG1229 (*Labrenzia* sp.), which had shown modest growth on DMSP at 1 mmol dm^{-3} over 10 days, no real change in cell number was detected during the 72 h time course experiments. Despite this, the cells still showed rapid removal of DMSP via both assimilatory and dissimilatory pathways, but in contrast to DG1236, proportionally more DMSP was diverted through the dissimilatory pathway in the absence of glucose. This result suggests that without glucose, DG1229 did not

require as much sulfur to meet its cellular growth demand and thus it dissimilated the DMSP to DMS, possibly generating acrylate, which it could then use as a sole carbon source (Table 1). It is difficult to reconcile the differences in how glucose affects the fate of DMSP by DG1229 and DG1236. It does suggest that the two organisms may have relatively different metabolisms. The markedly higher rate of DMS oxidation in DG1229 may reflect a propensity for sulfur oxidation by the *Labrenzia*, as another dinoflagellate-associated species, *Labrenzia. alexandrii* DFL-11, was shown to oxidise thiosulfate, producing energy that supported increased biomass production (Biebl et al. 2007). Ecologically, while *Labrenzia* (formerly *Stappia*) are not uncommon associates of algae (Weber and King 2007; Green et al. 2004; Biebl et al. 2007), they are markedly less abundant than other members of the *Roseobacter* clade (Green D, unpubl. data). So while their ecological relevance is questionable, this study represents the first demonstration that they too can metabolise DMSP.

It has previously been demonstrated that the stage of an algal bloom may have a significant influence on the relative amount of DMS detected (Ledyard and Dacey 1996; Turner et al. 1988). Data suggest that as a bloom develops, DMS production from DMSP may be relatively low, however, DMS consumption rates are proportionally lower, allowing DMS concentrations to build up. This may occur because the total DOM produced by actively growing phytoplankton can be relatively low. As the bloom declines (cell senescence, viral lysis, grazing pressure), there is potential for large quantities of DMS to be produced, but this production can be met by a near equal rate of consumption (Archer et al. 2002; van Duyl et al. 1998; Zubkov et al. 2004). This can result in a decoupling between the concentrations of DMSP and DMS, which is reflected in studies showing poor correlation between the two compounds in many marine systems (Liss et al. 1997). Evidence from our study and others (e.g., del Valle et al. 2007) suggest that the majority of the DMS consumption may be via the oxidation pathway and that this pathway will be strongly influenced by the availability of alternative carbon sources (Green et al. 2011; Vila-Costa et al. 2006). This hypothesis is supported by results from research in the Equatorial Pacific studying high and low productivity regions around the Galapagos Islands (Hatton et al. 1998); a regime where increased productivity was supported by enhanced nutrient availability in the upwelling to the west of the islands. Results showed that increased productivity was reflected in enhanced concentrations of particulate DMSP (on average 50% more) to the west of the Islands. This increase in DMSP, though not apparent in the concentration of DMS, was reflected in the concentrations of DMSO with an average of 32 nmol dm^{-3} to the west of the island and 9 nmol dm^{-3} to the East. More significantly, the ratio of dissolved DMSO to particulate DMSP was 0.42 in the lower productivity region, but was 1.05 in the higher productivity region. Here the dissolved DMSO concentration was actually in excess of DMSP (Hatton et al. 1998). The specific inference being that higher productivity equates to higher DOM production, which may be used by the bacterioplankton community as the necessary carbon source to drive the increased rates of DMS oxidation observed in these regions.

This pattern has also been observed in both mesocosm (Mogg et al., unpublished data) and phytoplankton studies (Simó et al. 1998; Hatton and Wilson 2007), which show high particulate DMSP concentrations associated with early bloom and stationary phase growth, but a shift towards higher DMSO concentrations, with an increase in the ratio of dissolved DMSO to particulate DMSP from $\sim$0.1 to $\sim$0.6 as the cells go into senescence. These studies had hypothesised that the increase in dissolved DMSO may result from increased intracellular concentrations of DMSO, produced as a product of nutritive or oxidative stress, or as a part of either the antioxidant cascade (Sunda et al. 2002) or sulfur overflow mechanism (Stefels 2000). An alternative hypothesis to the above is that late log phase growth and senescence are associated with increased cell permeability and leakage of intracellular contents, increasing both the level of DMSP and DOC available at the algal cell surface for DMS oxidation to DMSO. Therefore, our results highlight that the role algae may play in the production of DMSO may need to be re-evaluated. It is vital that we pull apart the relative contribution of the algal cell directly from the associated bacteria if we are to understand and model the processes governing the cycling and production of DMS in marine systems. Work is currently underway in our laboratory to investigate the relative contribution of algae and associated bacteria to the concentration of DMSO seen in algal cultures and incubation studies (Hatton et al., in prep).

Overall, the results from this study clearly demonstrate that many different bacterial types can oxidise DMS to DMSO, especially when an additional energy source is available, and supports the hypothesis that bacterial oxidation of DMS to DMSO is a significant consumption pathway for DMS in natural marine systems. Furthermore our results strongly point to the concentration of DMSP and biological availability of DOC in the field as key parameters affecting the sea surface DMS concentration, and we speculate that knowing the DOC concentration will help to better predict the flux of DMS from sea surface.

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