Coupling of Dimethylsulfide Oxidation to Biomass Production by a Marine Flavobacterium

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Dimethylsulfide (DMS) is an important climatically active gas. In the sea, DMS is produced primarily by microbial metabolism of the compatible solute dimethylsulfoniopropionate. Laboratory growth of Bacteroidetes with DMS resulted in its oxidation to dimethyl sulfoxide but only in the presence of glucose. We hypothesized that electrons liberated from sulfur oxidation were used to augment biomass production.

The gas dimethylsulfide (DMS) is regarded as an important volatile sulfur species because, in the atmosphere, its oxidation products are postulated to influence large-scale climate regulation through enhanced cloud formation (5). The production of DMS from marine sources has been calculated to represent a significant proportion (ca. 50 to 84%) of its total global production (2, 30). While terrestrial DMS production is primarily industrial in origin, marine DMS comes principally from microbial breakdown of the compatible solute dimethylsulfoniopropionate (DMSP) (20). However, only a relatively small fraction of the total DMS produced escapes to the atmosphere (3, 10, 21), with the majority being turned over by biological processes in a matter of days (e.g., 0.6 to 4.6 days [see reference 21]).

At present, the primary processes driving DMS removal are believed to be photochemical oxidation (15) and bacterial consumption (21). While microbial utilization of DMS has been demonstrated in several groups of C1-utilizing marine organisms, such as Methylophaga (8, 25, 27), it is still unclear how important these organisms are to the removal of DMS in the surface ocean. Instead, a growing body of evidence shows that under certain circumstances, much of the DMS removed (e.g., 81 to 93% [7]) is not consumed for carbon or sulfur by bacteria but rather ends up in a pool of dissolved nonvolatile sulfur composed of dimethyl sulfide (DMSO) and sulfate (7, 22, 27, 32). Thus, there is still uncertainty about the processes responsible for DMS removal from the surface ocean, and this needs to be resolved if we are to be able to predict DMS concentrations as a result of changing environmental conditions.

DMS is oxidized to DMSO by diverse members of the Bacteroidetes. The oxidation of DMS to DMSO by bacteria was first demonstrated during the photoautotrophic growth of purple sulfur bacteria (14, 28, 31). Later work showed that DMS oxidation also occurs during chemoheterotrophic bacterial growth (17). While DMS oxidation has been suggested as a likely property of many marine bacteria due to the ubiquitous occurrence of DMS in the ocean (14), surprisingly few bacteria typical of productive surface ocean waters have been shown to oxidize DMS. Sagittula stellata E-37 is an exception (12), as it is a member of the Roseobacter clade that can be abundant in productive surface waters (4). Importantly, the work of Vila-Costa and colleagues (27) implicated a wider range of bacteria as being capable of DMS turnover. They identified Methyllophaga predominantly in DMS-enriched mesocosm experiments conducted using Sargasso Sea and Gulf of Mexico seawater, but they also detected Alphaproteobacteria and several members of the Flavobacteria (Bacteroidetes), whose abundances were stimulated by DMS enrichment.

It was during work with the cultivable bacteria isolated from the DMSP-producing dinoflagellate Scripsiella trochoidea CCAP 1134/1 that we observed that a wide range of these isolates were capable of DMS oxidation (A. D. Hatton et al., submitted for publication). Notably, the rates of DMS oxidation were typically higher for the Bacteroidetes isolates. We reasoned that as Flavobacteria are ubiquitous and can comprise up to ~40% of total bacterial abundance in surface waters (1, 9, 11), this group of organisms could be important to the turnover of DMS.

To test this hypothesis, we examined five Bacteroidetes strains to establish whether DMS oxidation was a common phenotype of marine Bacteroidetes (Table 1). Four strains were isolated from the dinoflagellate Scripsiella trochoidea CCAP 1134/1, and Leeuwenhoekiella blandensis MED217 was isolated from Mediterranean seawater (23). The isolates were initially grown to stationary phase in ZM/10 broth, a dilute yeast extract-peptone seawater medium (13), and then washed and suspended in 1.5 ml of basal seawater medium supplemented with Fe-EDTA and vitamins (12) and seeded in 6-ml crimp-top glass vials with polytetrafluoroethylene (PTFE)-lined stoppers to which ca. 1.5 µM DMS was added in the presence or absence of 5 mM glucose. All samples were incubated in the dark (ca. 21°C) to minimize photochemical oxidation of DMS. Samples were removed periodically for DMS and DMSO analysis by pulse flame photometric gas chromatography (GC) as described previously (15, 16).

The results showed that after 72 h of incubation, the Bacteroidetes strains had removed between 46 and 97% of the DMS but only in the presence of glucose (Table 1). If glucose was
omitted, relatively little (0 to 14%) of the DMS was removed. On average, ~90% of the DMS removed could be quantita-
tively accounted for as DMSO (Table 1). Thus, diverse mem-
bers of the Bacteroidetes (Flavobacteria and Sphingobacteria)
are capable of DMS removal via its oxidation to DMSO. How-
ever, the fact that the presence of glucose was essential for a
substantive amount of the DMS to be oxidized suggested that
this process was, potentially, cometabolism. Following Dalton
and colleagues’ redefinition of cometabolism (6), we hypothe-
size that glucose, as the “growth substrate,” provides reducing
equivalents via its catabolism for the oxidation of DMS, the
“nongrowth substrate.”

**DMS oxidation is contingent on an available source of glu-
cose.** To examine the requirement of glucose for DMS oxida-
tion, we tested various glucose concentrations on DMS re-
moval and DMSO formation by one strain of Flavobacteria,
DG1233. This strain was chosen because it grew well on glu-
cose in basal seawater medium, and it was typical of other
Flavobacteria in our collection. The methodology was imple-
mented as described above, but glucose concentrations varied
between 0 and 5 mM. This experiment showed that glucose
concentrations ≥250 μM had a significant effect (Student’s t
test, α = 0.01) on the rate and amount of DMS oxidized to
DMSO (Fig. 1). Lower concentrations of glucose (≥25 μM)
had relatively little effect on the total amount of DMS oxidized.
For these lower glucose concentrations, it was noted that after
24 h, all growth (A540) had ceased (data not shown). This was
assumed to be due to the complete utilization of glucose as the
sole available carbon source; thus, we infer that there were
insufficient reducing equivalents available for DMS oxidation.
Overall, the interpretation was that glucose catabolism pro-
duced a source of reducing equivalents that was essential for
DMS cometabolism by strain DG1233.

**DMS oxidation can be coupled with biomass production.**
The oxidation of DMS to DMSO will yield 2 electrons, which
can be coupled with protein and biomass formation in purple
sulfur bacteria (14, 28, 31). As Bacteroidetes belong to a sepa-
rate phylum, we sought to understand whether they could also
couple electron flow from DMS oxidation with biomass pro-
duction. To test this question, we grew DG1233 in 1.5 ml of
basal medium containing 5 mM glucose in crimped 6-ml serum
vials as described above and supplemented each bottle with
DMS at concentrations ranging between 0 and 34 mM. At time
points up to 96 h, triplicate vials of each DMS treatment were
sacrificed for DMSO measurement, cell turbidity, and total
protein assay. Samples for DMSO analysis were frozen imme-
diately and stored (−20°C) until analyzed by GC as described
above. Cell turbidity was measured immediately using a UV–
visible-light (Vis) spectrometer (A540), and the bacterial cells
were harvested by centrifugation (13,000 × g for 30 min),
suspended in sterile deionized water, and recentrifuged to col-
lect the washed cells. The cell pellets were then stored frozen
(−20°C) until protein was assayed using the bicinchoninic acid
(BCA) protein assay (26).

Growth of DG1233 with increasing concentrations of DMS
resulted in increasing amounts of DMSO being formed (Fig.

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**TABLE 1. DMS oxidation by marine Bacteroidetes isolates**

<table>
<thead>
<tr>
<th>Bacteroidetes strain</th>
<th>Without glucose</th>
<th>With glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% DMS removed</td>
<td>% DMSO formed</td>
</tr>
<tr>
<td><em>Flavobacteria, Flavobacteriaceae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Maricula</em> sp. strain DG1233</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Arenibacter</em> sp. strain DG1238</td>
<td>13.9</td>
<td>54.4</td>
</tr>
<tr>
<td><em>L. blanden&quot;as</em> MED217</td>
<td>1.4</td>
<td>100</td>
</tr>
<tr>
<td><em>Sphingobacteria, Flammeovirgaceae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Reichenbachia</em> sp. strain DG1392</td>
<td>5.8</td>
<td>100</td>
</tr>
<tr>
<td><em>Roseivirga</em> sp. strain DG1392</td>
<td>85.8</td>
<td>100</td>
</tr>
</tbody>
</table>

* a Strains were incubated with ~1.5 μM DMS with or without 5 mM glucose.
* b Taxonomy is based on the affiliation of 16S rRNA gene sequences as determined by the RDP II Classifier program (29). Accession numbers for strains DG1232 to DG1392 are DQ486479, DQ486480, DQ486485, and DQ486489, respectively.
* c Percentage of DMS removed after 72 h in the presence or absence of 5 mM glucose.
* d Percentage of removed DMS that was quantitatively recovered as DMSO.

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**FIG. 1. DMS oxidation by DG1233 in the presence of increasing concentrations of glucose.** DMS removal (A) and concomitant DMSO formation (B) at each glucose concentration. ●, no glucose; ○, 1 μM glucose; ▽, 25 μM glucose; △, 250 μM glucose; ■, 5 mM glucose. Error bars denote 1 standard deviation (SD) of the mean of results from triplicate samples.
increasing DMS concentrations. It showed that the amount of biomass formed increased with concentrations, which are potentially high on or around algal surfaces and particles (24). Such variable enzymatic efficiencies may reflect the involvement of different enzyme systems, or it may reflect organisms’ adaptation to niches of differing DMS concentrations. Furthermore, as DMS is ubiquitous throughout the world’s surface ocean, the observations of this study suggest that DMS oxidation could be an important mechanism by which marine bacteria can augment their cellular energy requirements. Nevertheless, Flavobacteria are ecologically important in productive waters, where they are frequent colonizers of both living and decaying particulate organic matter (1, 9, 11). Therefore, it is highly likely that this association with organic-rich material will bring Flavobacteria into contact with elevated concentrations of both DMS and labile organic matter, the result of which can be the cometabolism of DMS generating an electron flow that can be utilized for cellular energetics, such as biomass formation. Furthermore, as DMS is ubiquitous throughout the world’s surface ocean, the observations of this study suggest that DMS oxidation could be an important auxiliary energy source for Bacteroidetes and implies that DMS oxidation can be a readily available source of energy for DG1233.

Overall, this study suggests that DMS cometabolism is likely to be an important auxiliary energy source for Flavobacteria and that it may be widespread among other Bacteroidetes. However, this study clearly cannot infer the significance that DMS cometabolism has on bacterial growth and energetics in the field, as the concentrations tested here were orders of magnitude higher than the ca. 5 nM mean seawater DMS concentration to which these organisms are typically exposed. Nevertheless, Flavobacteria are ecologically important in productive waters, where they are frequent colonizers of both living and decaying particulate organic matter (1, 9, 11). Therefore, it is highly likely that this association with organic-rich material will bring Flavobacteria into contact with elevated concentrations of both DMS and labile organic matter, the result of which can be the cometabolism of DMS generating an electron flow that can be utilized for cellular energetics, such as biomass formation. Furthermore, as DMS is ubiquitous throughout the world’s surface ocean, the observations of this study suggest that DMS oxidation could be an important mechanism by which marine bacteria can augment their cellular energy requirements.

References


