Release of Dimethylsulfide from Dimethylsulfoniopropionate by Plant-Associated Salt Marsh Fungi

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The range of types of microbes with dimethylsulfoniopropionate (DMSP) lyase capability (enzymatic release of dimethylsulfide [DMS] from DMSP) has recently been expanded from bacteria and eukaryotic algae to include fungi (a species of the genus *Fusarium* [M. K. Bacic and D. C. Yoch, Appl. Environ. Microbiol. 64:106–111, 1998]). Fungi (especially ascomycetes) are the predominant decomposers of shoots of smooth cordgrass, the principal grass of Atlantic salt marshes of the United States. Since the high rates of release of DMS from smooth cordgrass marshes have a temporal peak that coincides with peak shoot death, we hypothesized that cordgrass fungi were involved in this DMS release. We tested seven species of the known smooth cordgrass ascomycetes and discovered that six of them exhibited DMSP lyase activity. We also tested two species of ascomycetes from other DMSP-containing plants, and both were DMSP lyase competent. For comparison, we tested 11 species of ascomycetes and mitosporic fungi from halophytes that do not contain DMSP; of these 11, only 3 were positive for DMSP lyase. A third group tested, marine oomycetes (four species of the genera *Halophytophthora* and *Pythium*, mostly from mangroves), showed no DMSP lyase activity. Two of the strains of fungi found to be positive for DMSP lyase also exhibited uptake of DMS, an apparently rare combination of capabilities. In conclusion, a strong correlation exists between a fungal decomposer's ability to catabolize DMSP via the DMSP lyase pathway and the host plant's production of DMSP as a secondary product.

Traditionally, the only components of the dimethylsulfide (DMS) emission process in the marine environment studied seriously by biological oceanographers were the phytoplankton and macroalgae (1, 3, 6, 14, 18, 19). Over the past few years, the roles of bacteria (11, 12, 20, 28), protozoa (microzooplankton), and metazoans (50, 52, 54) have been viewed as important to this process. More recently it was discovered that a fungus, *Fusarium lateritium*, also degrades dimethylsulfoniopropionate (DMSP; $[\text{CH}_3]_2\text{S}^+-\text{CH}_2-\text{CH}_2-\text{COO}^-$) and releases DMS (2, 5). The enzyme responsible for this degradation is DMSP lyase, and its activity in a fungus adds another major phylogenetic group to the participants in the DMS-emitting portion of the global sulfur cycle. This DMSP lyase-producing fungus was isolated from the salt marsh ecosystem, where more DMS is emitted per unit area than from any other ecosystem (44). DMS emissions from the salt marsh occur mainly over the areas populated by cordgrasses, such as *Spartina alterniflora* (44), which contains high DMSP levels (80 to 280 $\mu\text{mol g}^{-1}$ [dry weight]) (9, 27, 41). While the details of the DMS emission process in salt marshes remain unclear, it is known that the rate of emissions increases during leaf decay (9). Furthermore, it has been well established that the main mediators of cordgrass shoot decay are fungi (32). These *Spartina* decomposers contribute to nutrient cycling in the salt marsh via lignocellulolysis and immobilization of plant nitrogen during the decay process (32, 33). Although it has been suggested that lignocellulolytic fungi are capable of using organic material found in living *Spartina* shoots (33), no studies have been done to investigate the possibilities that these fungi also degrade DMSP and cause the increased DMS emissions

during leaf senescence and decay. This paper reports the distribution of DMSP lyase-producing fungi associated with DMSP-producing, halophytic plants in the salt marsh and suggests that fungi play a significant role in DMS emissions in decaying cordgrass.

MATERIALS AND METHODS

Isolates of fungi and oomycetes. Nineteen species of true fungi (eumycetes; kingdom *Fungi*) and four species of oomycetes (species of the genus *Halophytophthora*; kingdom *Protocista* or *Chromista*) (Table 1) were obtained from ascospores, conidia, or zoospores isolated from decaying salt marsh grass or mangrove leaves (25). All of the fungi obtained from marsh grasses were tested for DMSP lyase activity immediately following isolation (except *Phaeosphaeria spartinicola* from *Spartina anglica*, isolated in 1991, and *Buergenerula spartinae* I, isolated in 1982). The oomycetes and fungi from mangrove and other tree leaves (i.e., the mangroves *Rhizophora mangle*, *Rhizophora stylosa*, and *Avicennia germinans* and wild cherry, *Prunus serotina*) were isolated from 1985 to 1996 and maintained on pieces of autoclaved natural substrate atop dilute (2% [vol/vol]) V-8 agar (25), with transferring no more than twice per year.

All of the isolates from *S. alterniflora* (smooth cordgrass) were from marshes of Sapelo Island, Ga., except those with an "FSU" suffix (Table 1). The FSU isolates were from a site near the Florida State University Marine Laboratory on the Gulf of Mexico coast of St. George Sound, Fla. The *P. spartinicola* from *S. anglica* was collected alongside Dungarvan Harbour, Ireland. The *Pleospora spartinae* from *Spartina foliosa* (California cordgrass) was collected from San Francisco Bay, Calif. The fungal isolates from *Juncus roemerianus* (black needle rush), *Spartina patens* (salt meadow cordgrass), and *Distichlis spicata* (salt grass) and the oomycete (*Halophytophthora vesicula*) from *P. serotina* (wild cherry) were from Sapelo Island marshes. The three mitosporic fungal species from mangroves were from Miami, Fla. (*Dendryphiella salina*); Quepos, Costa Rica (*Zalerion varium*); and Yap Island, Caroline Islands (*Cirrenalia basiminuta*). The *Lulworthia* sp. was from Ft. Pierce, Fla. The species of the genus *Halophytophthora* from mangroves were from the Bahama Islands, except for *H. vesicula* I (Miami, Fla.) and *Halophytophthora exoprolifera* II (Harrington Sound, Bermuda).

Screening for fungal DMS emissions. The fungi and oomycetes were inoculated into a basal salt medium (2) containing either 1 mM DMSP or DMSP supplemented with either 2 mg of β -sitosterol (Sigma) per liter alone or β -sitosterol and 20 mg of yeast extract per liter. The fungi and oomycetes were inoculated on slants of this medium in 15-ml vials that were sealed with Teflon-coated rubber caps and aluminum crimps once the fungi had begun to grow into

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TABLE 1. DMSP lyase activity in fungi and oomycetes isolated from various marine plant species

Plant host	Fungal inhabitant ^a	DMSP lyase activity ^b
DMSP producing		
<i>Spartina alterniflora</i>	<i>Phaeosphaeria spartanica</i> SAP (A)	10.4 ^c
	<i>Phaeosphaeria spartanica</i> FSU (A)	15.9
	<i>Mycosphaerella</i> sp. II strain SAP (A)	6.6 ^c
	<i>Mycosphaerella</i> sp. II strain FSU (A)	ND ^d
	<i>Buergenerula spartinae</i> I (A)	3.2 ^c
	<i>Buergenerula spartinae</i> II (A)	8.9 ^c
	<i>Phaeosphaeria halima</i> (A)	5.9
	<i>Passeriniella obiones</i> II (A)	11.4 ^c
	<i>Passeriniella obiones</i> III (A)	6.0 ^c
	<i>Lachnum spartinae</i> I (A)	0.0
	<i>Lachnum spartinae</i> II (A)	0.0
	<i>Phaeosphaeria neomartima</i> I (A)	3.3
	<i>Phaeosphaeria neomartima</i> II (A)	40.7 ^c
<i>Spartina anglica</i>	<i>Phaeosphaeria spartanica</i> IRE (A)	10.5
<i>Spartina foliosa</i>	<i>Pleospora spartinae</i> (A)	9.3
Non-DMSP producing		
<i>Juncus roemerianus</i>	<i>Aropsiclus junci</i> (A)	0.0
	<i>Papulosa amerspora</i> (A)	0.0
	<i>Loratospora aestuarii</i> I (A)	0.0
	<i>Loratospora aestuarii</i> II (A)	0.0
	<i>Tremateia halophila</i> I (A)	3.1
	<i>Tremateia halophila</i> II (A)	2.3
	<i>Massarina ricifera</i> (A)	0.0
<i>Spartina patens</i>	<i>Phoma</i> sp. (M)	7.5
<i>Distichlis spicata</i>	<i>Phaeosphaeria</i> sp. (A)	0.0
<i>Rhizophora mangle</i>	<i>Pythium grandisporangium</i> (O)	0.0
	<i>Halophytophthora vesicula</i> (O)	0.0
	<i>Halophytophthora spinosa</i> I (O)	0.0
	<i>Halophytophthora spinosa</i> II (O)	0.0
	<i>Halophytophthora exoprolifera</i> I (O)	0.0
	<i>Halophytophthora exoprolifera</i> II (O)	0.0
	<i>Zalerion varium</i> (M)	0.0
	<i>Lulworthia</i> sp. (A)	0.0
	<i>Dendryphiella salina</i> (M)	ND
<i>Rhizophora stylosa</i>	<i>Cirrenalia basiminuta</i> (M)	0.0
<i>Avicennia germinans</i>	<i>Halophytophthora vesicula</i> (O)	0.0
<i>Prunus serotina</i>	<i>Halophytophthora vesicula</i> (O)	0.0

^a A, ascomycete; M, mitosporic (conidial) fungus; O, oomycete.

^b Specific activity for DMSP lyase is expressed as nanomoles of DMS per minute per milligram of protein. Values are the means of two experiments.

^c DMSP lyase was induced by glycine betaine as well as DMSP.

^d ND, not done. For fungal isolates that took up DMS, due to the rapid disappearance of DMS after addition of DMSP, we were not able to determine lyase-specific activity.

the agar. Gas samples (50 μ l) from these vials were removed 4, 5, 6, and 8 days after inoculation and injected into a gas chromatograph (GC-8A flame ionization detector; Shimadzu) to test for the presence of DMS (11). *F. lateritium* cultures (2) and uninoculated media served as positive and negative controls, respectively. The initial screening of fungal species for DMSP lyase activity was performed in a blind assay in which the individual performing the analysis was unaware which strains came from DMSP-producing plants.

DMSP lyase activity. To challenge the screening results for those strains testing positive for DMSP lyase activity, cultures were incubated in liquid medium (tryptic soy broth [TSB] diluted 1:1 with seawater and containing 25 μ g of chloramphenicol ml⁻¹) for 1 to 2 weeks in order to obtain sufficient biomass for the assays. During the growth period, the cultures were regularly checked for the presence of bacteria by plating out aliquots onto tryptic soy agar. For routine DMSP lyase assays, fungi were washed to remove all traces of medium and resuspended in buffer (50 mM PO₄ [pH 7.0] diluted 1:1 in seawater). To vent any ethanol produced by these strains (ethanol produces a peak on our gas chromatograph close to that of DMS), it was necessary to incubate them for 1 h, and then rinse and resuspend the biomass in buffer. DMSP (1 mM) was added to these fungal suspensions and DMS emissions were monitored. Suspensions with

no added DMSP served as controls; this control was particularly important because fungi can produce and release DMS via pathways that do not include cleavage of DMSP (46).

Acrylate mineralization. The fungi were inoculated onto slants of the basal salt medium (2) containing 20 mg of yeast extract per liter plus or minus 5 mM acrylate in 15-ml vials that were sealed with Teflon-coated rubber caps and aluminum crimps once slight growth was apparent. After 1 week of incubation, gas samples were removed and carbon dioxide was analyzed on a Carle Instruments gas chromatograph as described previously (13). Integrator counts of CO₂ from vials containing only yeast extract were subtracted from those supplemented with acrylate before conversions to molar concentrations of CO₂ were calculated. One-milliliter aliquots of analytical grade CO₂ served as standards.

DMSP lyase induction. Either DMSP or glycine betaine (2 mM) was added to buffered cell suspensions. After 1.5 h, the fungal mycelia were filtered, thoroughly washed, and resuspended in fresh buffer and 1 mM DMSP was added as a substrate to measure the rate of lyase activity. DMS emissions were monitored every 15 min for 2 h.

Protein. At the end of each experiment the fungal biomass from each vial was removed by filtration, dried, put into 1 ml of 2 M NaOH, and boiled for 5 min. Protein was estimated with the Bradford reagent (BioRad, Hercules, Calif.).

DMSP uptake. To test all the strains shown in Table 1 for DMS uptake activity, DMS was added to 1 ml of buffered mycelial suspensions in 15-ml vials, which were immediately sealed. DMS was allowed to equilibrate with the liquid phase for 1 h, and then gas samples were removed every 2 h and analyzed. The two fungi, *Mycosphaerella* sp. II strain FSU and *D. salina*, which evolved DMS from added DMSP and took up DMS were further analyzed. Because a good kinetic analysis of DMS emission and DMSP uptake was difficult to obtain with these strains, a sulfur balance experiment was performed. DMSP (2 mM) was added to buffered suspensions of the fungi, and DMS emissions were monitored every 15 min until the DMS in the gas phase disappeared. Three hours after DMS emissions ceased, the fungi were separated from the suspension medium by filtration and 4 M NaOH was added to both fractions to completely cleave any remaining DMSP to DMS. DMS was then measured in both the fungal fraction and the medium as a way of quantitating DMSP.

Chemicals. DMSP was synthesized from DMS and acrylate according to the method of Chambers et al. (7). DMSP was standardized against the pure commercial reagent obtained from Research Plus, Bayonne, N.J. Acrylate and DMS were obtained from Aldrich, Milwaukee, Wis. Glycine betaine was purchased from Sigma.

RESULTS

DMSP lyase producers. The correlation between fungi which have DMSP lyase activity and their plant origins is seen in Table 1. Seventeen of the 36 strains tested had DMSP lyase activity, and most of these (13 of 17) were residents of DMSP-producing plants. Among decomposers of *S. alterniflora* tested, only the two isolates of *Lachnum spartinae* (strains I and II) had no DMSP lyase activity. A principal fungal inhabitant of dead leaves of this grass, the ascomycete *P. spartanica*, occupying an average of 89% of the leaf area (36), was able to degrade DMSP with the subsequent release of DMS. None of the oomycetes tested had DMSP lyase activity, and they exhibited no growth on medium containing DMSP alone. All DMS emissions from these fungal suspensions were dependent on added DMSP. Aliquots of the fungal and oomycete suspensions were plated onto nutrient agar after the experiment to ensure that a pure culture was involved. In all cases, only the species of interest was present. DMSP lyase-specific activity for all fungal isolates except two is shown in Table 1. Different fungi had markedly different enzyme activities, with the highest activity being found in a *S. alterniflora* dweller, *Phaeosphaeria neomartima* II, and the lowest activity being found in isolates of *Tremateia halophila*, which live on a non-DMSP-producing plant. The specific activities for DMSP lyases from *Mycosphaerella* sp. II strain FSU and *D. salina*, which were obtained from *S. alterniflora* and *R. mangle* (red mangrove), respectively, were not determined because the DMS produced was simultaneously taken up by these fungi (see below).

DMSP lyase induction. Two general patterns of DMSP lyase induction, each represented by a number of the fungal species, are indicated in Table 1. All DMS-producing isolates were induced by DMSP, but some were also induced by glycine betaine. Kinetics representative of all species having DMSP

FIG. 1. Kinetics of DMSP lyase activity by the marine fungus *B. spartinae* II. These results are representative of those fungi whose DMSP lyases were inducible by DMSP alone or by either DMSP or glycine betaine. The glycine betaine-inducible strains are identified in Table 1. At time zero, 1 mM DMSP was added to washed cell suspensions that were either uninduced (■) or induced with 2 mM DMSP (●) or 2 mM glycine betaine (▲). This experiment was repeated twice for each fungal strain, and the data shown here are representative of the results.

lyase activity following induction are shown in Fig. 1. *B. spartinae* (and the other species identified in Table 1, footnote c) was induced by both DMSP and glycine betaine. The following additional observations were made (data not shown) concerning the induction of DMSP lyase by various fungal species: (i) *Phaeosphaeria halima*, a *S. alterniflora* inhabitant, appeared to express DMSP lyase activity constitutively; (ii) in both strains of *Passeriniella obiones*, glycine betaine induced DMSP lyase faster than did DMSP; (iii) *P. spartinae*, *Phoma* sp., *Tremateia halophila*, and *B. spartinae* required a longer (4-h) induction period before enzyme activity was seen; and (iv) preincubation with glycine betaine seemed to delay (by ~1 h) enzyme induction by DMSP in *P. spartinicola*.

DMS uptake. During the DMSP lyase induction experiments, it was noted that when DMSP was added to suspensions of *Mycosphaerella* sp. II strain FSU and *D. salina*, they produced a small amount of DMS, which rapidly disappeared. It appeared that these strains were utilizing the DMS they had just produced. Thus, the fungi were tested for DMS uptake, and of all the fungal isolates seen in Table 1, only *Mycosphaerella* sp. II strain FSU and *D. salina* had this activity. Figure 2 shows the kinetics of the DMS uptake experiments for *Mycosphaerella* sp. II strain FSU; *D. salina*, at equivalent protein concentrations, showed similar results. Therefore, these fungi not only degrade DMSP, releasing DMS, but also recycle the DMS produced. The addition of 5 μg of nystatin ml⁻¹ completely inhibited the DMS uptake system, suggesting an active (energy-dependent) process.

In studying DMS evolution and its concomitant uptake by these strains, it was noted that DMSP uptake continued after DMS had reached its maximum in the gas phase and had disappeared. Table 2 shows the fate of DMSP added to suspensions of these two fungi. Of the 2 mM DMSP added to the cultures, only about 2% was emitted to the gas phase as DMS. Part of the DMSP taken up was found to be stored intracellularly; at the end of 3 h, *Mycosphaerella* and *D. salina* contained 8 and 26% of the added DMSP, respectively. Approximately one-quarter (31 and 24%, respectively) of the DMSP remained unused in the suspending buffer. Thus, a large percentage, 40 and 60%, respectively, of the total added DMSP remained unaccounted for and was metabolized without first being emitted as DMS. Since these strains have observable lyase activity and no other gases (such as methanethiol [48])

FIG. 2. DMS utilization by *Mycosphaerella* sp. II strain FSU. DMS was added to cell suspensions at time zero, and its disappearance was monitored by gas chromatography. *Mycosphaerella* suspensions contained 5 μg of nystatin ml⁻¹ (■) or no addition (●). A suspension of *P. halima*, representing those fungi that do not take up DMS, served as a control (▲). The data presented are representative of two experiments.

were detected following the disappearance of DMS, the unaccounted for DMSP is listed in Table 2 as "putative metabolized DMS."

Acrylate mineralization. All species of fungi having DMSP lyase activity (Table 1) were tested for their ability to grow on and mineralize acrylic acid (CH₂=CH₂COO⁻). All DMSP lyase-containing species, including the two which also utilized DMS, grew on the acrylate and oxidized it to CO₂ (range = 0.5 to 2.6 μmol of CO₂ produced bottle⁻¹ day⁻¹ [mean = 1.6]).

DISCUSSION

DMS emission to the atmosphere has the potential to lead to changes in the albedo effect and thereby to influence global climatic patterns (8). Reported rates of emission of DMS from smooth cordgrass (*S. alterniflora*) salt marshes range up to tens of micromoles m⁻² h⁻¹ (31), greater than rates of emission from noncordgrass marshes or through the air-water interface above suspended phytoplankton (26, 31). The shoots of smooth cordgrass, which, when living, contain high levels of DMSP (up to >100 μmol g⁻¹ [41]), are the principal sources of DMS from the cordgrass marsh (9). Rates of DMS release from smooth cordgrass shoots peak in September and October (45), at the time when the death rate of smooth cordgrass leaves is also at a maximum (10, 38, 42).

TABLE 2. Fate of DMSP in the fungal strains that took up DMS^a

DMSP allocation	% Total DMSP added to fungi ^b	
	<i>Mycosphaerella</i>	<i>Dendryphiella</i>
Emitted as DMS	1.8	2.5
Intracellular ^c	8.1	26.4
In buffer (unused) ^c	30.7	23.9
Putative metabolized DMS	61.3	49.7

^a DMSP (2 mM) was added to cultures, and after 3 h, a mass balance was carried out.

^b The values represent the means of two experiments. The mean values for protein were as follows: *Mycosphaerella*, 0.23 mg ml⁻¹ and *Dendryphiella*, 0.46 mg ml⁻¹.

^c Measured as DMS following base (NaOH) cleavage of the DMSP that remained after 3 h.

The autumnal maxima for DMS release and the rate of appearance of standing dead shoot parts suggest that the standing dead shoot parts are the source of DMS (9). *S. alterniflora* could possess DMSP lyase activity of its own, so the release may be partly due to activity of a DMSP lyase in senescent shoots, but an alternative or additional explanation is now available: the ascomycetous-fungal decomposers of standing cordgrass shoot parts, strong secondary producers within the standing dead material (37), are responsible for the breakdown of cordgrass DMSP via fungal DMSP lyase activity. Several of the smooth cordgrass ascomycetes in Table 1 (i.e., *P. spartinicola*, *B. spartinae*, and *P. obiones*), with substantial DMSP lyase activity (3 to 16 nmol of DMS min⁻¹ mg of fungal protein⁻¹), are among the established secondary producers (40). The rates of DMSP lyase activity that we observed for mycelium grown in rich, proteinaceous liquid medium could be low compared to rates for mycelium inside the natural solid substrate. The fact that we observed growth rates of fungi on solid DMSP medium that were approximately fourfold higher than those on liquid medium suggests that our measured rates of DMSP lyase activity in liquid may also be low. DMSP would be expected to be present primarily in the cell cytoplasm (16), and early in their digestion of cordgrass tissue, cordgrass ascomycetes completely destroy the cytoplasmic contents of leaf cells (see Fig. 2 of reference 40). The fact that decomposer fungi of DMSP-containing plants have the potential to be involved in release of DMS from dead plant material has implications beyond the salt marsh ecosystem, e.g., for sugarcane fields (41a).

Given the distinct possibility that salt marsh ascomycetes cause the release of a portion of the DMS from cordgrass shoots, it is possible that the methods used to measure DMS release from cordgrass marshes have yielded underestimates of natural release rates. The activity of cordgrass ascomycetes is strongly dependent on the water content of standing decaying cordgrass shoots (39). In order to measure DMS release from marshes, chambers have been placed over cordgrass shoots (9, 31, 45). Dacey et al. (9) pointed out that the enclosure chambers could cause inaccuracies in DMS flux estimates due to CO₂ stripping. Although the chambers would be expected to maintain high internal humidity, they could also prevent water contact (e.g., tides, rain, fog, and dew) with dead shoot material, thereby potentially limiting the rate of fungal DMSP lyase activity. Note that in the work of Morrison and Hines (31), when sediments were flooded, DMS rates increased. This increase was attributed to deterrence of DMS flux to sediments by the water cover (9, 31), but the increase could also have been partially due to tidal wetting of the least-elevated decaying blades, permitting enhanced fungal activity.

We screened only one oomycete (*H. vesicula*) from the salt marsh ecosystem (the strain from *P. serotina* leaves [Table 1]). The logic for this limited screening was that oomycetes do not appear to have a substantial presence in decomposing leaves of marsh grasses (33). Neither this salt marsh oomycete nor any of the other, mangrove ecosystem oomycetes that we screened exhibited DMSP lyase activity (Table 1). This finding might be viewed as counterintuitive, since the oomycetes have an evolutionary lineage that associates them with microalgae (15), some species of which are well established producers of DMSP and DMSP lyase (1, 19). However, since (i) the oomycetes are believed to be best adapted to growth in fallen leaves (especially those of mangroves) (33), (ii) at least one mangrove species (*R. mangle*) has been found not to accumulate DMSP in its leaves (9), and (iii) oomycetes commonly utilize proline as the compatible solute when faced with conditions of low

water potential (30), perhaps the absence of DMSP lyase in marine oomycetes is to be expected.

Most of the fungi in which we detected DMSP lyase activity are known to inhabit only the decaying parts of shoots of the DMSP-containing plants listed in Table 1. One exception to the restriction to DMSP-producing hosts may be *P. neomaritima*, which may also occur in non-DMSP-producing grasses (23). However, in a recent exhaustive examination of a reported non-DMSP-containing host, *J. roemerianus*, Kohlmeyer et al. (reference 24 and references therein) did not find *P. neomaritima*. A second exception is *D. salina*, a common inhabitant of intertidally deposited marine substrates of many kinds, including drift seagrasses and macroalgae (34, 35, 43), which are known producers of DMSP (9, 16). The two additional exceptions are the *Phoma* sp. and *T. halophila* that we obtained from the non-DMSP-producing grasses *S. patens* and *J. roemerianus*, respectively. The explanation for these two exceptions is not obvious. Since DMSP is a substrate analog of other readily usable carbon sources found in non-DMSP-producing grasses, the possibility exists that this lyase acts upon other substrates.

Only one of our screened fungal species that are restricted to decomposition of standing dead smooth cordgrass was found not to possess DMSP lyase: *L. spartinae* (Table 1). One potential reason for this might be that *L. spartinae* is found on older decaying leaf sheaths (4); it may be that by the time that *L. spartinae* is most active, succeeding other leaf sheath species such as *P. neomaritima*, all DMSP from the dead leaf sheaths has been lost. Furthermore, *L. spartinae* is an apothecial ascomycete, a taxon quite distinct from those of the other *S. alterniflora* and *S. foliosa* ascomycetes, which are pseudothecial or perithecial ascomycetes (17).

The utilization of DMSP by suspensions of the fungi *Mycosphaerella* sp. II FSU and *D. salina*, and the subsequent appearance and disappearance of the DMS from these cultures (Table 2), appears to be the first evidence of DMSP lyase and DMS uptake activities occurring in the same organism. Presumably the DMS is utilized as an additional carbon and energy source by these cells. We have not, however, studied DMS utilization by these fungi in the absence of DMSP catabolism. Both DMSP catabolism to DMS and DMS uptake are common phenomena in natural populations from sea and estuarine waters (21, 47, 51, 53) and salt marsh sediments (22). However, these environments contain complex microbial populations, and it cannot be determined if any of their members are capable of both activities. Furthermore, there is no indication from the literature that any of those microbes whose DMSP lyase has been studied in pure culture can also take up DMS (11, 12, 29, 49). The results of the acrylate mineralization test showed that these two fungi utilize not only DMS but also the other product of DMSP lyase activity, acrylate (data not shown).

The DMSP lyase activity of the salt marsh plant fungal inhabitants ranged from 3 to 41 nmol of DMS min⁻¹ mg of cell protein⁻¹. These values are lower than those seen in bacteria, where they range from 100 to 750 nmol of DMS min⁻¹ mg of cell protein⁻¹ when assayed under comparable conditions (11, 12, 55). Other values for bacterial DMSP lyase exist in the literature, but these rates were calculated on a per-cell basis (28, 29, 54). The fungus *F. lateritium*, whose DMSP lyase we recently characterized (2), had activities that were generally higher than those of the plant-associated species reported here; values of the former ranged from 20 to 80 nmol of DMS min⁻¹ mg of cell protein⁻¹. We must emphasize again that the relatively lower values we report here for fungal DMSP lyase activity were from cells grown in rich, proteinaceous liquid

medium and may be lower than those that are possible on solid natural substrata. *S. alterniflora* is reported to have 5 to 50 μmol of DMSP g (fresh weight) of tissue⁻¹ (reference 16 and references therein), which, after several assumptions have been made, can be calculated to be approximately 5 to 50 mM DMSP in the cytosol, concentrations far above those required for optimal activity of *F. lateritium* (2) and presumably of the *Spartina* decomposers listed in Table 1.

Our finding that DMSP lyase activity in salt marsh plant fungal decomposers was highly correlated with DMSP production by the plants suggests that an evolutionary relationship may exist between these plants and the decomposer fungi they harbor. That is, the fungi may have developed the lyase concomitantly with the salt marsh plants' acquiring the ability to synthesize DMSP. Alternatively, those decomposer species that have the ability to degrade the large supply of cellular DMSP could have an adaptive advantage over non-DMSP lyase-producing fungi for this niche in the *Spartina* leaf. Consistent with both hypotheses is the fact that the *Spartina* DMSP lyase producers are also capable of utilizing the resulting acrylate.

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