

# DMSP LYASE IN MARINE MACRO- AND MICROALGAE

## Intraspecific Differences in Cleavage Activity

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### SUMMARY

The enzymatic cleavage of dimethylsulfoniopropionate (DMSP) to dimethylsulfide (DMS) was investigated in twenty-one strains of marine macro- and microalgae, representing seven algal classes. The enzymes involved in this cleavage are DMSP lyases, producing DMS from DMSP. All algal strains tested were able to synthesize and accumulate various levels of intracellular DMSP but only twelve strains showed DMSP lyase activity. It was possible to identify subgroups of strong and weak DMS producers. The first subgroup included three *Enteromorpha* species (*E. clathrata*, *E. intestinalis*, *E. compressa*) and *Phaeocystis* sp. with specific activities in crude cell extracts ranging from 7 to over 100 nmol DMS min<sup>-1</sup> (mg cell protein)<sup>-1</sup>. The second subgroup was composed of a sub-antarctic strain of *Acrosiphonia arcta*, *Polysiphonia lanosa*, two strains of *Emiliana huxleyi*, *Acrosiphonia sonderi*, *Ulva lactuca* and *Enteromorpha bulbosa*. In this subgroup activity ranged from 0.01 to 0.2 nmol DMS min<sup>-1</sup> (mg cell protein)<sup>-1</sup>. No DMSP lyase was detectable in a sub-arctic strain of *Acrosiphonia arcta*, *Acrosiphonia sonderi*, *Monostroma arcticum*, *Prasiola crispa*, *Polysiphonia urceolata*, *Ascoseira mirabilis*, *Laminaria saccharina* and *Tetraselmis subcordiformis*. Non-optimal assay conditions and bacterial contamination may have affected rates in some samples, but the results suggest the widespread presence of DMSP lyase among algal taxa, and also raises the possibility that closely-related species may have quite different lyase activities or function.

### INTRODUCTION

The spontaneous breakdown rate of dissolved dimethylsulfoniopropionate (DMSP) to dimethylsulfide (DMS) and acrylic acid is known to be very slow in seawater (3). Since DMS is the dominant sulfur gas found in marine surface waters (1), the understanding of biological processes leading to DMS production is of major importance.

Presently, it is believed that there are two major biological mechanisms whereby DMS is produced in aerobic seawater: microbial (primarily bacterial) activity may cleave dissolved DMSP (free DMSP in the seawater) or particulate DMSP (from decaying algal material and fecal pellets) (4, 16), and enzymes associated with or released from algal cells may cleave the DMSP to DMS (2; 23). The enzymes involved in this process are DMSP lyases, a group of carbon-sulfur lyases, which are classified as dimethylpropiothetin-dethiomethylases. Several reports of bacterial DMSP lyases have examined the production of DMS in seawater and bacterial cultures (6, 15, 19). Recently, a DMSP lyase from a DMS producing marine bacterium was purified and characterized (5).

DMSP is a prominent sulfur compound in various groups of algae (12, 14) but not all taxa produce and accumulate DMSP intracellularly in amounts high enough to be quantified by existing methods. The intracellular concentrations of DMSP vary widely in individual species. Even within one species, the concentrations may be subject to physiological or environmental control (7; 8; 11; see also Keller and Korjeff-Bellows, this volume). Although much is known about the occurrence of DMSP in algae, little is known about the ability of algae to degrade DMSP. The Rhodophytes *Polysiphonia lanosa* (2) and *Polysiphonia paniculata* (21), the Prymnesiophyte *Phaeocystis sp.* (23), and the Chlorophyte *Enteromorpha clathrata* (Steinke and Kirst, in press) are the only currently known algal species to exhibit DMSP lyase activities. Since extracts of *Cryptothecodinium (Gyrodinium) cohnii*, a heterotrophic dinoflagellate, showed DMS production due to lyase activity (10), there is evidence that dinophytes may also be able to cleave DMSP.

In this study we used an enzyme assay developed for DMSP lyase measurements in *Enteromorpha clathrata* (Steinke and Kirst, in press) to screen twenty-one strains of marine macro- and microalgae for the ability to enzymatically cleave DMSP to DMS. The data presented provide a preliminary overview on DMSP lyase distribution in different classes of marine algae.

## MATERIALS AND METHODS

### Plant Material and Culture Conditions

The algae used in this study originated from different sources and were maintained under conditions listed in Table 1. All cultures were unialgal with the exception of *Scrippsiella sp.* (Dinophyceae). This culture was contaminated with a small (< 10  $\mu\text{m}$ ) chlorophyte flagellate.

The macroalgae were kept in 500 or 1000 ml glass beakers, containing 0.2  $\mu\text{m}$  filtered North Sea water. The seawater was aerated, enriched with nutrients (PES enrichment; 22) and adjusted to a salinity of 32 practical salinity units (PSU; equivalent to parts per thousand) with distilled water. This culture medium was changed at least once a month to avoid nutrient limitation. Light was supplied by fluorescent tubes at a light-dark rhythm of 18:6 hours.

Microalgae were cultivated in Erlenmeyer flasks using three types of media (Table 1), adjusted to a salinity of 32 to 34 PSU. *Phaeocystis sp.* cultures were placed on a gyratory shaker at 80 rpm and consisted of non-colonial, single cells when harvested. The culture with *Tetraselmis subcordiformis* was aerated, whereas *Emiliania huxleyi*, *Scrippsiella sp.* and *Calciodinellum operosum* were manually stirred once a day. Light was supplied by fluorescent tubes at a light-dark rhythm of 18:6 hours.

Table 1. Origin and culture conditions of algal strains.

Class	Species	Origin of isolate		Culture conditions			
		Strain #	Location and year of collection	Temp.	Light	Medium <sup>a</sup>	
<b>Macroalgae</b>							
Chlorophyceae	<i>Enteromorpha clathrata</i>	1086	Disko-Island (Greenland) 1990	0	15	NS (PES)	
	<i>Enteromorpha intestinalis</i>	1088	Disko-Island (Greenland) 1990	0	11	NS (PES)	
	<i>Enteromorpha bulbosa</i>	1002	King-George-Island (Antarctica) 1986	0	13	NS (PES)	
	<i>Enteromorpha bulbosa</i>	1002	King-George-Island (Antarctica) 1986	5	35	NS (PES)	
	<i>Enteromorpha compressa</i>	1134	Kongsfjord (Svalbard) 1991	5	25	NS (PES)	
	<i>Acrosiphonia arcta</i>	1120	Disko-Island (Greenland) 1990	5	33	NS (PES)	
	<i>Acrosiphonia arcta</i>	1071	King-George-Island (Antarctica) 1987	5	26	NS (PES)	
	<i>Acrosiphonia sonderi</i>	1130	Kongsfjord (Svalbard) 1991	5	27	NS (PES)	
	<i>Acrosiphonia sonderi</i>	1132	Kongsfjord (Svalbard) 1991	5	29	NS (PES)	
	<i>Monostroma arcticum</i>	1139	Kongsfjord (Svalbard) 1991	5	27	NS (PES)	
	<i>Prasiola crispa</i>	1040	King-George-Island (Antarctica) 1986	5	34	NS (PES)	
	Ulvoiphyceae	<i>Ulva lactuca</i>	1128	Disko-Island (Greenland) 1990	5	35	NS (PES)
		Rhodophyceae	<i>Polysiphonia urceolata</i>	2093	Disko-Island (Greenland) 1990	5	21
<i>Polysiphonia lanosa</i>	Poly M		Belfast (Ireland) 1994	10	6	NS (PES)	
Phaeophyceae	<i>Ascoseira mirabilis</i>	3068	King-George-Island (Antarctica) 1987	0	6	NS (PES)	
	<i>Laminaria saccharina</i>	3123 & 3124	Kongsfjord (Svalbard) 1991	5	32	NS (PES)	
<b>Microalgae</b>							
Prasinophyceae	<i>Tetraselmis subcordiformis</i>	161-1a	New Haven, CT (USA) 1952	25	80	AQUIL (f/2)	
Prymnesiophyceae	<i>Phaeocystis</i> sp.	Phaeo B	North Sea (Germany)	10	60	AQUIL (K)	
	<i>Emiliania huxleyi</i>	Emil D	North Sea (Norway)	15	70	ANT (f/50)	
	<i>Emiliania huxleyi</i>	G 1779	(oceanic)	15	70	ANT (f/50)	
Dinophyceae	<i>Scrippsiella</i> sp.	Scripps		20	70	WIMEX (f/2)	
	<i>Calciodinellum operosum</i>	Calcio 74	Naples (Italy) 1995	20	70	WIMEX (f/2)	

a) Various media and additional enrichments were used for the cultivation of algae: NS = North Sea water; AQUIL = artificial seawater (prepared after 20); ANT = antarctic water; WIMEX = commercial seasalt mixture; PES = Provasoli enrichment factor (prepared after 22); K = K-enrichment factor (prepared after 13); f/2 and f/50 = dilutions of the f-enrichment factor (prepared after 9).

## Preparation of Extracts

**Macroalgae:** 1 to 2 g fresh weight of algal tissue was briefly rinsed in ice-cold distilled water to remove external salts and carefully blotted dry, then homogenized with a pestle and mortar under liquid nitrogen. All subsequent preparations were carried out at 4°C unless stated otherwise. The homogenate was suspended in 3 ml extraction buffer containing 100 mM 2-[N-morpholino] ethanesulfonic acid (MES), 13 mM calcium chloride dihydrate, 10% (v/v) glycerol and 0.5% (v/v) detergent (Tween 80; polyoxyethylenesorbitan monooleate), adjusted with NaOH to pH 6.2. The extract was incubated on ice for 30 min to dissolve membranes, followed by centrifugation three times for 5 min at 1200 x g. The supernatant was collected and the pellet resuspended in 2 ml extraction buffer after each centrifugation step. The supernatants were pooled and centrifuged 5 min at 4000 x g. The liquid phase of the crude cell-free extract was used in the enzyme assay to test for DMSP lyase activity.

**Microalgae:** Cultures were harvested during late exponential growth phase (6 to 700 10<sup>6</sup> cells, depending on the strain investigated) by centrifugation at 3500 x g for 10 min at 4°C. The culture with *Scrippsiella sp.* was centrifuged at 700 x g for 5 min at 4°C. Most (80%) of the contaminating flagellates remained in the supernatant and were removed by this procedure. The centrifuged microalgae were resuspended in extraction buffer (described above) and homogenized with a French Press at 138 MPa (equivalent to 20,000 psi). Microscopic observation indicated that >90% of cells were destroyed during this step. The resulting crude extracts, containing cell debris, were incubated on ice for 30 min to allow membranes to dissolve. No attempt was made to further purify the extracts because microalgal biomass was low compared to macroalgal samples.

Macro- and micro-algal crude extracts were purged of gaseous DMS (derived from enzymatic conversion of cellular DMSP) with compressed air for 30 min at 0°C. 1-ml aliquots of the purged crude extracts were stored in Eppendorf micro test tubes at -80°C to be assayed for DMSP lyase activity the day following the extraction. This allowed simultaneous assay of many extracts, following their preparation. Protein concentrations were determined after all enzyme assays were carried out (see below). Another 500 µl subsample was heated for 60 min at 95°C to destroy enzymatic activity and this was used as a control.

## Enzyme Assays

Prior to the enzyme assay, the extracts of *Enteromorpha clathrata*, *Enteromorpha intestinalis*, *Enteromorpha compressa* and *Phaeocystis sp.* were diluted by 1:100 with extraction buffer to reduce DMSP lyase activity in the assay. 10-µl extraction buffer containing 30 mM dithiothreitol (DTT) was transferred into a gas tight screw-capped glass vial (volume 1.2 ml) and equipped with a Teflon-coated silicone septum. The potential production of DMS from algal DMSP was assayed after adding 270 µl of the extract to the test buffer after incubating for 10 min at 27°C. Usually, only small amounts of DMS were detectable in the extracts, but the extract of *Scrippsiella sp.* produced large amounts (see results). After this pre-test for DMS, the reaction was started by adding 20 µl extraction buffer containing dissolved DMSP to a final concentration of 2 mM. The DMSP used in all experiments was prepared according to Larher *et al.* (18). 100 to 300 µl of the gas phase (headspace) was sampled over 10 to 60 minutes, and injected with a gas-tight syringe into a Shimadzu 9 A gas chromatograph equipped with a FPD detector. Operating conditions are given in Karsten *et al.* (12). The detection limit was about 8 pmol DMS, or approximately 80 nM DMS in a 100 µl headspace sample. Rates were calculated as the difference in DMS concentrations before DMSP addition, and after incubation with DMSP. Production rates in heated controls, when observed, were subtracted to give enzyme activities. Specific DMSP

lyase activities were then calculated based on protein concentration (see following section) in the algal extracts.

### Protein Determinations

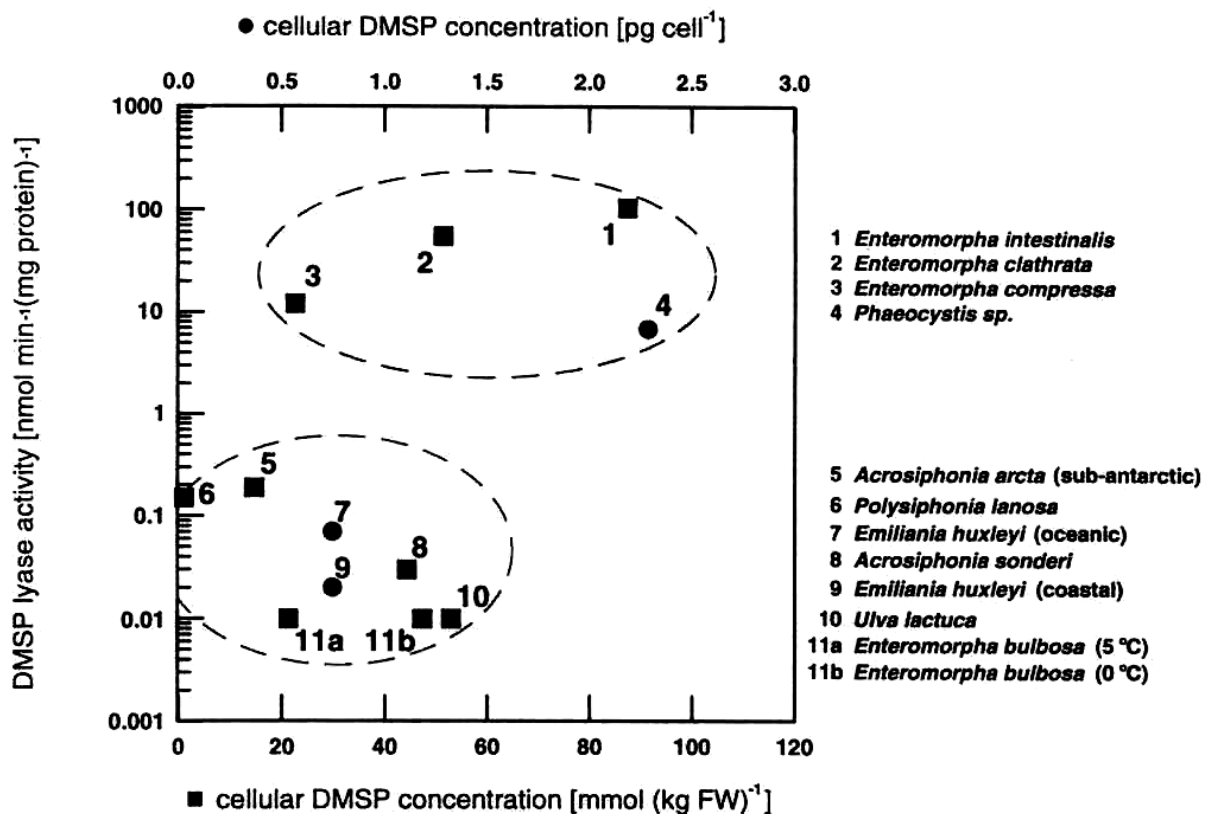
Protein concentration in the extracts was determined spectrophotometrically at 750 nm using a commercial test assay (Bio-Rad detergent compatible protein assay). Relative absorption was quantified by comparing with a series of protein standards with known concentrations of bovine serum albumin in extraction buffer.

### DMSP Determinations

The intracellular DMSP concentrations in the microalgae (*Tetraselmis sp.* clone OPT4, *Phaeocystis sp.* clone 677-3 and *Emiliania huxleyi* clone BT6) were taken from the literature (14) and therefore, do not exactly match strains investigated in this study. The macroalgal DMSP concentrations were determined according to the method described by Karsten *et al.* (12).

## RESULTS AND DISCUSSION

All algal species tested were able to synthesize and accumulate DMSP intracellularly. The range of this accumulation varied greatly from 0.01 to 87 mmol (kg fresh weight)<sup>-1</sup>



**Figure 1.** DMSP lyase activities vs. intracellular DMSP concentrations in various algal strains. DMSP concentrations for microalgae (●) are expressed as pg cell<sup>-1</sup> on the top x-axis (data from 14) and for macroalgae (■) as mmol (kg freshweight)<sup>-1</sup> on the bottom x-axis. See text for strains without DMSP lyase activity detectable.

(macroalgae) or 0.75 to 2.29 pg cell<sup>-1</sup> (microalgae). Initially, we choose *Ascoseira mirabilis* and *Laminaria saccharina* (both Phaeophyta) as non-DMSP containing controls but traces of DMSP (DMS released upon NaOH treatment) were detected in both species (0.07 mmol DMSP (kg freshweight)<sup>-1</sup>).

Despite the presence of DMSP in all strains tested, only twelve of twenty-one strains investigated in this study showed measurable DMSP cleavage by means of DMSP lyases. There was no relationship between DMSP lyase activity and intracellular DMSP concentration of the species investigated (Figure 1).

There appeared to be three groups that showed different levels of DMSP lyase activity. In one group no DMSP lyase was detectable (e.g. DMS production in the samples did not exceed that in heated controls). This group included a sub-arctic strain of *Acrosiphonia arcta*, *A. sonderi* (strain 1132), *Monostroma arcticum*, *Prasiola crispa*, *Polysiphonia urceolata*, *Ascoseira mirabilis*, *Laminaria saccharina* and *Tetraselmis subcordiformis*. Another group was composed of weak DMS producers. The species in this group showed activities ranging from 0.01 to 0.2 nmol DMS min<sup>-1</sup> (mg cell protein)<sup>-1</sup>. A sub-antarctic strain of *Acrosiphonia arcta*, *A. sonderi* (strain 1130), *Polysiphonia lanosa*, both strains of *Emiliania huxleyi*, *Ulva lactuca* and *Enteromorpha bulbosa* belonged into this group. A final group of strong DMS producers showed production rates at least two orders of magnitude higher, from 7 to over 100 nmol DMS min<sup>-1</sup> (mg cell protein)<sup>-1</sup>. This group was represented by three *Enteromorpha* species (*E. intestinalis*, *E. clathrata* and *E. compressa*) and *Phaeocystis* sp. The strain of *Scrippsiella* sp. appeared to have strong DMSP lyase activity as well. However, we could not quantify production because we were not able to reduce initial DMS concentrations - which arose from the enzymatic conversion of DMSP readily available in the crude extract. This endogenous DMS production made it impossible to quantify any additional DMS production which might have occurred due to the experimental addition of 2 mM DMSP.

It is likely that the distinction between weak and strong producers is to some extent an artefact of our small sample size. We expect that other strains might yield rates in between these two groups, giving a continuum of rates. In fact, *C. operosum* showed a DMSP lyase activity of 2 nmol DMS min<sup>-1</sup> (mg cell protein)<sup>-1</sup>. It is not included in figure 1 because no estimate of intracellular DMSP concentration was available from the literature.

It is also likely that measured lyase activities were affected by our assay conditions. As already pointed out, we used an enzyme assay which was optimized to test for DMSP lyase in *Enteromorpha clathrata*. Requirements for optimal function of DMSP lyases in other species may be significantly different. For example, different pH optima are reported for several DMSP lyases. The pH optimum for the DMSP lyase from *Polysiphonia lanosa* was 5.1 (2), whereas that from *Cryptothecodinium cohnii* was 6 to 6.5 (10), similar to the pH optimum in *Enteromorpha clathrata* (Steinke and Kirst, in press). It is highly likely that our assay conditions were not optimal for some of the weak DMS producers, yielding underestimates of their rates. We also froze algal extracts prior to assay, so that many extracts could be prepared and then tested simultaneously. It is possible that the lyase activities in some species were reduced by cold storage, while others were not. However, our assay conditions were mild, so it is unlikely our underestimates were drastic. We also emphasize the dramatic differences between the strong and weak producers - over 100-fold change in activity - rather than the specific rates. Nonetheless, reaction conditions should be adjusted optimally for each organism tested, and these data must be regarded as preliminary until further tests can be conducted. We note, however, that three species of *Enteromorpha* showed high activities, but *E. bulbosa* was a very weak DMS producer. This suggests, despite the limitations of our method, that closely related species may either have very different concentrations of DMSP lyase, or functionally different enzymes which may require different assay conditions.

While non-optimal assay conditions may have yielded underestimates of the rates in the group of weak lyase activity, bacterial contamination by DMSP-lysing strains could have resulted in overestimates, or false positives. Our algal material was not axenic, but before enzyme extractions, cultures were checked by light microscopy for bacterial contamination. In all algal cultures numbers of bacteria were low. Nonetheless, high bacterial lyase activities could have contributed significantly to DMS production, especially among the weak producers. Using the  $v_{\max}$  of  $1.09 \mu\text{mol DMS min}^{-1} (\text{mg cell protein})^{-1}$  for the DMSP lyase of strain M3A, an *Alcaligenes*-like bacterium (5), and an approximate protein concentration of  $70 \mu\text{g cell protein per } 10^9 \text{ cells in M3A}$  (De Souza, pers. comm.) the numbers of bacteria necessary to generate the low activities measured in the group of weak DMS producers may be calculated:  $1.5 \times 10^6$  to  $3 \times 10^6 \text{ cells ml}^{-1}$  of strain M3A would be necessary to explain the activities found in this group. We believe such a density would have been detected by microscopy. Furthermore, rinsing the algal tissue in distilled water and blotting it dry probably reduced the number of bacteria attached to the surface of the algal thalli. We therefore believe that bacterial contribution to our results was, in most cases, modest. However, in the strains of *Emiliania huxleyi* which tested positive, concentrating the cultures by centrifugation may have resulted in bacteria numbers high enough to explain the DMSP lyase activity measured.

## CONCLUSION

The results of this study, though preliminary, suggest that DMSP lyase is probably widespread among marine algae and that levels of DMSP lyase activity show high intraspecific variability. It is also likely that related strains may show very different DMSP lyase activities, as observed for *Enteromorpha* spp. Whether these differences are due to enzyme concentration, enzyme expression, functional enzyme variation, or environmental cues, we cannot yet say.

Some of the observed variability is undoubtedly due to non-optimal assay conditions, or possible contamination by bacteria. Ideally, all assays should be optimized for each strain, and conducted on axenic material. This is an extreme undertaking and few such studies on comparative enzymology exist even for well-characterized enzymes. We wish to emphasize not the specific rates, but rather the broad pattern of algal DMSP lyase distribution and variability. We believe that algal DMS production should receive more attention because it has been shown that bacterial degradation of extracellular, dissolved DMSP produces relatively little DMS (17) and demethylation of dissolved DMSP may be a more important sink for DMSP than cleavage to DMS (24). Therefore, even slow conversion of the intracellular DMSP pool by algal enzymes might contribute significantly to DMS production.

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