S. A. Van Bergeijk · L. J. Stal

Dimethylsulfoniopropionate and dimethylsulfide in the marine flatworm *Convoluta roscoffensis* and its algal symbiont

Abstract A distinct smell of dimethylsulfide (DMS) was noted at the edge of the intertidal mudflat of Marennes-Oléron Bay, at the French Atlantic coast, where dense populations of the marine flatworm *Convoluta roscoffensis* Graff (Platyhelminthes: Turbellaria) were present. DMS is the cleavage product of dimethylsulfoniopropionate (DMSP). DMSP was shown to be present in high amounts in sediment containing the flatworm as well as in axenic cultures of the symbiotic alga *Tetraselmis* sp. that was isolated from the flatworm. In untreated sediment samples containing *C. roscoffensis* the concentration of DMS was as high as ∼55 μmol l^{-1} sediment, and in samples that were fixed with glutaraldehyde the concentration of DMS was even three orders of magnitude higher (∼66 mmol l^{-1} sediment). This rapid cleavage of DMSP to DMS in fixed samples was unexpected. Pure DMSP was stable in glutaraldehyde, and it was therefore concluded that a DMSP-lyase was responsible for cleavage in the field samples. The isolated symbiotic alga, *Tetraselmis* sp., did not show DMSP-lyase activity, indicating that DMSP-lyase may have been present in the flatworm, although the role of bacteria could not be excluded. The Chl *a*-specific DMSP content of *C. roscoffensis* (∼200 mmol g^{-1}) was much higher than that of *Tetraselmis* sp. (∼30 mmol g^{-1}). Possibly, DMSP was not only present in the symbiotic alga, but was also incorporated in the body tissue of the flatworm. It remains unclear what the function of DMSP is in *C. roscoffensis*. In *Tetraselmis* sp., but not in *C. roscoffensis*, DMSP increased with increasing salinity. It was concluded that salinity probably does not play an important role in the dynamics of DMSP and DMS in sediment containing *C. roscoffensis*.

S. A. Van Bergeijk (✉)
IBED/Laboratory for Microbiology,
University of Amsterdam, Nieuwe Achtergracht 127,
1018 WS, The Netherlands

Tel.: +31-20-5257089; Fax: +31-20-5257064
e-mail: a417stef@chem.uva.nl

L. J. Stal
Netherlands Institute of Ecology,
Centre for Estuarine and Coastal Ecology,
P.O. Box 140, 4400 AC Yerseke, The Netherlands

Introduction

For almost a century the existence of a symbiosis between the marine flatworm *Convoluta roscoffensis* Graff and a potentially free-living unicellular green alga has been known (Gamble and Keeble 1903; Keeble and Gamble 1907). *C. roscoffensis* is found on sandy intertidal beaches on the south-east coast of Great Britain, the Channel Islands and the north-west coast of France (Keeble 1910; McFarlane 1982). Despite its restricted distribution, this symbiotic relationship has been relatively well studied.

The flatworms are 2–4 mm long and contain 2×10^4 to 7×10^4 endosymbiotic algal cells (Doonan and Gooday 1982). The symbiosis is established at each host generation. Juveniles, newly hatched from the egg, are aposymbiotic (free of algae), but they ingest algae of the prasinophycean genus *Tetraselmis* (Keeble and Gamble 1907; Douglas 1985). Inside the animal, the algae lose their pectinaceous cell wall ("theca"), flagella and eyespot and move to a sub-epidermal position in the worm, where they proliferate (Douglas 1983). Juveniles that do not succeed in establishing a symbiosis with algae do not mature and eventually die, whereas symbiotic adults do not ingest food and can live completely autotrophically in the light in seawater containing nitrate and phosphate (Holligan and Gooday 1975).

In the field, *Convoluta* colonies are easily recognized as deep green spots on the sand between the tide marks on the edge of residual seawater. The worms are phototactic and strongly gregarious. When the tide goes out the worms emerge from beneath the sand, forming dense colonies.

A feature of *C. roscoffensis* that has not been reported in the literature is a distinct smell coming from

dense patches of these organisms. This smell was noticed at the Marennes-Oléron Bay (Atlantic coast, France), where the present study was carried out. Preliminary measurements revealed that the smell was due to the presence of the volatile organic sulfur compound dimethylsulfide (DMS).

DMS is emitted from the marine environment to the atmosphere and plays a role in the global sulfur cycle (Lovelock et al. 1972; Chin and Jacob 1996) and in climatic forcing (Charlson et al. 1987; Ayers et al. 1997). The main precursor of DMS is dimethylsulfoniopropionate (DMSP), which is produced by a variety of marine phototrophic organisms (Keller et al. 1989; Karsten et al. 1996). DMSP can be cleaved to DMS and acrylate either chemically at high pH (Challenger et al. 1957; Dacey and Blough 1987; Visscher and Van Gemerden 1991) or enzymatically by DMSP-lyases, which are present in many marine macro- and microalgae (Steinke et al. 1996) and bacteria (Kiene 1990; De Souza and Yoch 1996).

Tetraselmis subcordiformis, a species closely related to the symbiotic alga in *C. roscoffensis*, is known to produce DMSP (Dickson and Kirst 1986). The DMSP content of *T. subcordiformis* has been found to increase with increasing salinity (Dickson and Kirst 1986), with decreasing temperature (Sheets and Rhodes 1996) and when the algae suffer from nitrogen limitation (Gröne and Kirst 1992).

In the present study we investigated whether the marine flatworm *C. roscoffensis*, collected at the Marennes-Oléron Bay, and the algal symbiont that we isolated from it, contained DMSP. We also tested if the algal symbiont was able to cleave DMSP to DMS by DMSP-lyase. During low tide, the populations of *C. roscoffensis* can be exposed to salinity changes due to freshwater runoff from land and rainfall or evaporation of water. Therefore, we investigated the effect of salinity on the production of DMSP and DMS by *C. roscoffensis* and by the algal symbiont. Furthermore, the effects of nitrogen deficiency and temperature on the DMSP production of the isolated alga was tested.

Materials and methods

Sampling

Samples were taken at low tide in the first week of November 1997, at the high water mark of the tidal mudflat of the Bay of Marennes-Oléron at the Atlantic coast (Charente-Maritime, France). The sampling site was characterized by an abrupt change from a fine muddy sediment on the mudflat to a very coarse sediment on the beach, which consisted of small pieces (∼0.2 cm) of broken shells. On the coarse sediment, along this clearly visible edge between the tide marks, dense colonies of *Convoluta roscoffensis* were present. A bulk sample of the upper layer of the sediment (∼0.5 cm) was sampled with a spoon and put in a small bucket.

To quantify the in situ DMS and DMSP content of the sediment containing the flatworm, we originally intended to use the standard method of sediment sampling using stainless steel cores, after which the sediment can be cut into horizontal slices. In the field, this

proved to be impossible due to the coarse, non-cohesive structure of the sediment and the constant rapid movement of the worms. Therefore, we decided to sample the worms from the thin layer of seawater overlying the sediment (=residual seawater). For this purpose we used a pipette to take ten replicate samples of 0.6 ml of seawater, which were put in 5-ml glass vials (Pierce). We could not control the number of worms these samples contained. To fix the samples, in order to prevent cleavage of DMSP to DMS, 2.4 ml of 0.5% glutaraldehyde solution [100 mM phtalate buffer, pH 4 (Jonkers et al. 1998)] was added, after which the vials were sealed gas-tight using Teflon-coated butyl rubber septa and aluminium crimp seal caps. These samples, together with the bulk sample which had been collected with a spoon, were taken to the laboratory. DMS was measured in the headspace of the seawater samples 7 h after sampling. Because the DMS concentration seemed exceptionally high, we took some additional samples from the bulk sample of sediment containing worms. Duplicate samples of 0.1 g of sediment were fixed in 3 ml of 0.5% glutaraldehyde solution or were put in 3 ml of seawater, for comparison. The sample vials were sealed as described above, and DMS was measured 0.5 h after sampling.

The rest of the bulk sample was used for isolation of the symbiotic alga and for the salinity experiment.

Isolation of the symbiotic alga

In order to isolate the symbiotic alga, some worms were squashed with a coverslip on a microscope slide and transferred to artificial seawater medium (ASN III, Rippka et al. 1979). For further cultivation and experiments, Kester medium was used (Kester et al. 1967). An axenic culture of the alga was obtained by washing ten times in sterile medium. Between the wash steps, the cell suspension was sonicated in an ultrasonic waterbath for 1 min and subsequently centrifuged at 770g for 10 min. The washed suspension of algae was plated on medium solidified with 1.5% agar. Axenic cultures were obtained by picking single colonies, which were inoculated in liquid medium. Axenity was checked by microscopy and by streaking on agar enriched with 0.5% glucose and 0.05% casamino acids. The isolated alga was identified as *Tetraselmis* sp.

Effect of salinity

Freshly collected worms were incubated at different salinities by putting 2.5 g of the sediment containing flatworms in 60-ml vials (Pierce) and adding 2.5 ml of aged (1 month), decanted seawater, to give a final volume of 3.5 ml. The seawater had a salinity of 32 PSU. A lower salinity (19 PSU) was obtained by the addition of demineralized water, and a higher salinity (40 PSU), by evaporation. Salinity was measured with a hand refractometer (Atago). The vials were sealed and incubated for 41.5 h in a climate chamber at 12 °C and a photon flux density of 20 μmol m^{-2} s^{-1}.

A similar experiment was done with *Tetraselmis* sp. Portions of culture (150 ml) were centrifuged aseptically at 770g for 10 min, and the pellets were resuspended in 50 ml of fresh medium (Kester et al. 1967) at different salinities. The salinity of the medium was adjusted by changing the concentrations of the major salts (NaCl, Na$_2$SO$_4$, MgCl$_2$, KCl and CaCl$_2$) proportionally. Portions of the algal suspensions (3.5 ml) were put in 60-ml vials (Pierce) which were sealed. The vials were incubated in a climate chamber at 25 °C and a photon flux density of 27 μmol m^{-2} s^{-1}.

During the experiments the formation of DMS was followed by analyzing the headspace at regular time intervals, and after terminating the experiments samples were taken for DMSP and Chl *a* analyses. Intracellular or particulate (DMSPp) and extracellular or dissolved DMSP (DMSPd) were separated by filtration of the samples over Whatmann GF/F filters using a Millipore filtration manifold. The filters were rinsed with medium or seawater in order to remove dissolved DMSP and put in 5-ml glass vials (Chrompack). Medium or seawater was added to a final volume equal to the original volume of the sample. Subsequently, NaOH (final conc. 2 M) was added, and the vials were closed immediately. The filtrate was treated similarly.

DMSP-lyase test

DMSP-lyase activity of the isolated *Tetraselmis* sp. was tested in crude cell extracts. *Tetraselmis* sp. was grown in an aerated 1-l Kluyver flask, at 25 °C and a photon flux density of 30 μmol m^{-2} s^{-1}. Wall growth was removed at regular intervals using a magnetic stirring bar inside the Kluyver flask. After 2 weeks, cells were harvested by aseptic centrifugation (770*g*, 10 min) and resuspended in buffer. Crude extracts were prepared in two different buffers, since the optimum conditions for a possible DMSP-lyase were unknown. The buffers used were 50 mM Tris, pH 8.5 with 5 mM dithiothreitol (Stefels and Dijkhuizen 1996) and 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.2 (Steinke et al. 1996). Crude extracts were prepared by disrupting the cells in a french pressure cell at 138 MPa, and were kept on ice. DMSP-lyase activity was measured as DMS formation in the headspace of sealed 10-ml vials (Pierce), after addition of 300 μM (final concentration) of DMSP to suspensions of the crude extracts in each of the two buffers. The assay was carried out at 20 °C and was stopped after 20 h. Different concentrations of crude extract (100–200 μg protein ml^{-1} assay mixture) were used, and controls were run that contained only buffer or buffer with heat-killed crude extract (60 min, 95 °C). The protein content of the extracts was measured by the Lowry method as described by Herbert et al. (1971), using bovine serum albumin as a standard.

Effect of temperature and nitrogen limitation

To test the effect of temperature and nitrogen limitation on the production of DMSP in *Tetraselmis* sp., a culture was grown for 10 days at 26 °C in an aerated 1-l Kluyver flask, divided in portions of 150 ml and centrifuged aseptically at 770*g* for 10 min. The pellets were washed and resuspended in fresh medium. Erlenmeyer flasks with 20 ml of algal suspension were incubated for 2 days at 6 °C and 26 °C. To test the effect of nitrogen limitation the algae at 26 °C were incubated in nitrogen-replete and nitrogen-deplete media. Nitrogen-replete medium contained 500 mg l^{-1} NaNO$_3$ and 0.36 mg l^{-1} (NH$_4$)$_3$Fe(III)C$_6$H$_5$O$_7\cdot$5H$_2$O. Nitrogen-deplete medium did not contain a nitrogen source. Photon flux density was 30 μmol m^{-2} s^{-1}.

Analyses of DMS and DMSP

DMS was measured in the headspace by taking 250 μl of gas sample with a gas-tight Hamilton syringe through the Teflon-coated butyl rubber septa. The samples were directly injected in a gas chromatograph (Chrompack CP 9000 or Shimadzu GC 14A), equipped with a wide bore column (Poraplot U, ID 0.53 mm, 25 m, Chrompack, The Netherlands) and a flame ionization detector. The temperatures of the detector, injector and oven were 200, 175 and 150 °C, respectively. The flows of air, H$_2$ and the carrier gas N$_2$ were 300, 30 and 8 ml min^{-1}, respectively. The retention time of DMS was approximately 2.5 min. The detection limit of this method was about 1 pmol per 250 μl of headspace injected.

DMSP was measured as DMS after alkaline hydrolysis. NaOH was added to the samples, which were incubated in the dark for at least 24 h. Standards of DMS (Merck) and DMSP were treated in exactly the same way as the samples. DMSP was synthesized according to Chambers et al. (1987).

Analysis of Chl *a* and cell number

Chl *a* was determined by extraction of the samples with *N,N*-dimethylformamide (De Winder et al. 1999). Samples of sediment containing *C. roscoffensis*, which had been incubated at different salinities, were stored at −20 °C and freeze-dried overnight before extraction of Chl *a*. Samples of cultures of *Tetraselmis* sp. were centrifuged at 2,800*g* for 10 min, and the pellets were stored at −20 °C until analysis.

Cells of *Tetraselmis* sp. were counted with a Coulter counter (Coulter Electronics, The Netherlands), equipped with a 50 μm aperture tube. Samples for cell counts were fixed with Lugol's fixative and stored in the dark at room temperature until they were counted. An average cell volume was obtained by measuring the cell diameter, with an eyepiece micrometer, of 30 cells of a batch culture of *Tetraselmis* sp. grown at 33 PSU. Cell volume was calculated using the formula $4/3(\pi r^3)$.

Results

Tetraselmis sp., isolated from *Convoluta roscoffensis*, contained approximately 45 fmol DMSP cell^{-1}, equivalent to 174 mM based on cell volume. Also *C. roscoffensis*, in symbiosis with *Tetraselmis* sp., had a high DMSP content. This was shown in sediment containing *C. roscoffensis* (Fig. 1B, C), but flatworms isolated from the sediment, present in the seawater overlying the sediment, had a high DMSP content as well. In field samples of residual seawater containing *C. roscoffensis*, which were fixed with 0.5% glutaraldehyde (phtalate buffer, pH 4), a very high concentration of DMS [2.6 (SD 2.1) mM] was measured 7 h after addition of the fixative. Addition of NaOH did not increase the DMS concentration in these samples. This suggested that DMSP present in the samples was rapidly cleaved to DMS as a result of fixation with glutaraldehyde. To confirm this, sediment samples (0.1 g) containing *C. roscoffensis* were incubated either in seawater or in 0.5% glutaraldehyde. Indeed, the DMS concentration in fixed sediment samples [26.5 (SD 18.0) μmol g^{-1} sediment $\approx$ 66.3 (SD 45.0) mmol l^{-1} sediment] was three orders of magnitude higher than in samples of sediment suspended in seawater [21.9 (SD 0.90) nmol g^{-1} sediment $\approx$ 54.8 (SD 2.30) μmol l^{-1} sediment], after 0.5 h of incubation. The worms were immediately killed by the fixative, but in seawater they stayed alive and were moving. Synthetic DMSP (2 mM) dissolved in 0.5% glutaraldehyde did not result in such a rapid formation of DMS (only 0.5% of DMSP was converted to DMS in 2 days). No DMSP-lyase activity was detected in a crude extract of *Tetraselmis* sp.

In cell suspensions of *Tetraselmis* sp. the particulate DMSP content was affected by salinity (Fig. 1E). At the start of the experiment, DMSPp in incubations at 21, 34 and 43 PSU was 121, 110 and 89.4 μM, respectively (not shown). After 41.5 h of incubation, DMSPp content was 121 (SD 3.50), 163 (SD 11.5) and 196 (SD 21.0) μM at 21, 34 and 43 PSU, respectively (Fig. 1E), indicating that net DMSP production occurred at 34 and 43 PSU, but not at 21 PSU. The amount of DMSP per cell showed the same response (Fig. 2A). Cell numbers of *Tetraselmis* sp. did not change during the experiment (Fig. 2B), neither did the protein or Chl *a* content (not shown). The dissolved DMSP and DMS concentrations in cell suspensions of *Tetraselmis* sp. were the same at all salinities (Figs. 1D, 3B). Since we could not detect DMSP-lyase in *Tetraselmis* sp., the DMS that was present in axenic cell suspensions of *Tetraselmis* sp. must
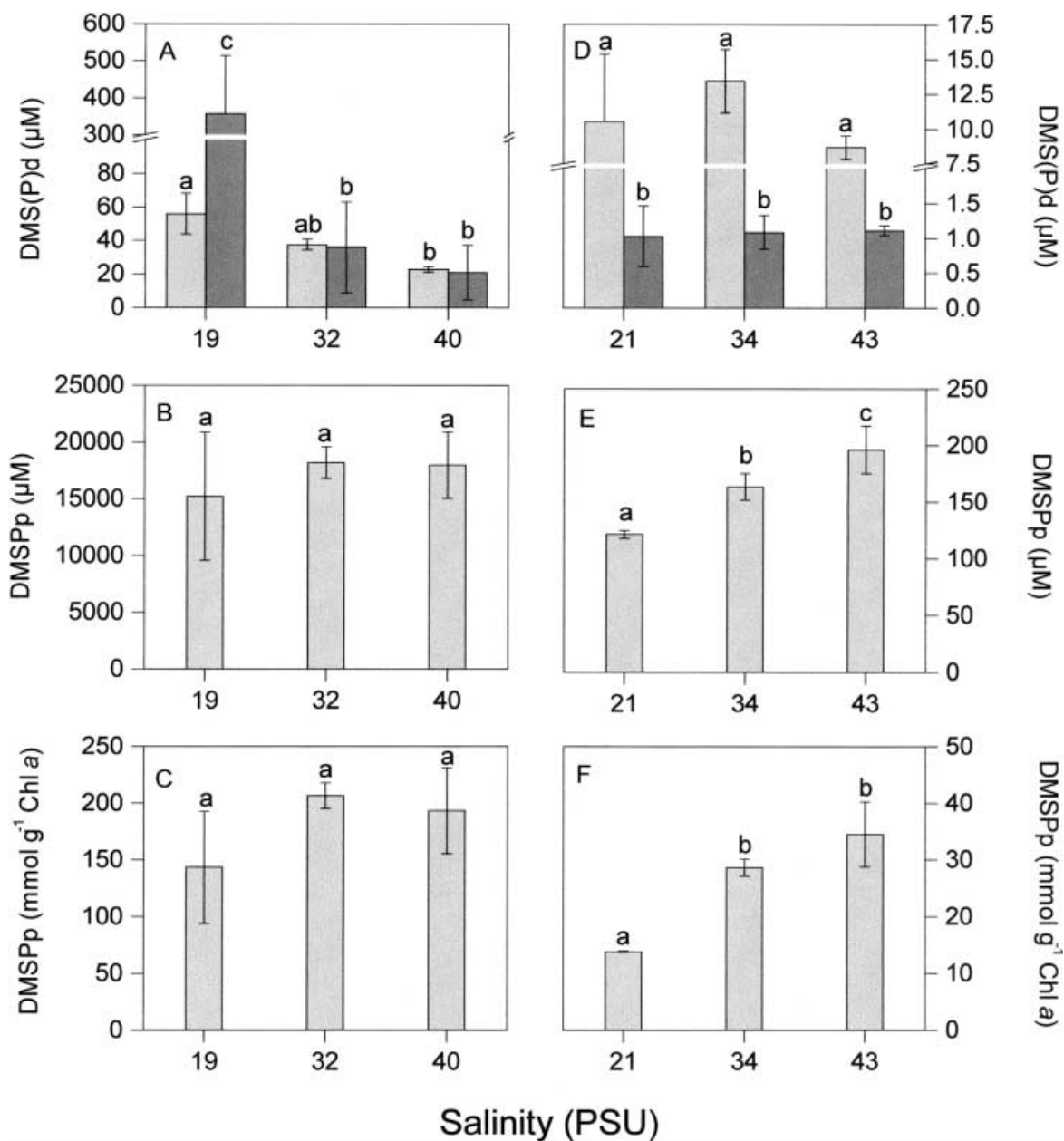
Fig. 1 *Convoluta roscoffensis* (**A, B, C**) and *Tetraselmis* sp. (**D, E, F**). Dissolved and particulate DMSP concentrations and DMSP to Chl *a* ratios in vials with sediment containing *C. roscoffensis* and with suspensions of *Tetraselmis* sp. after incubation at different salinities for 41.5 h. **A** and **D**: *light grey bars*: dissolved DMSP (DMSPd); *grey bars*: DMS concentration. **B** and **E**: particulate DMSP (DMSPp). **C** and **F**: Chl *a*-specific DMSP content. Error bars: SD of mean values of two (*C. roscoffensis*) or three (*Tetraselmis* sp.) replicate incubations. Initial value of DMSPp in vials with sediment containing *C. roscoffensis* was 14,668 ± 2,283.4 µM. Initial values of DMSPp in vials with suspensions of *Tetraselmis* sp. were 121, 110 and 89.4 µM at 21, 34 and 43 PSU, respectively. Significance of differences between means tested with two-way ANOVA, followed by paired comparison (Tukey HSD-test, $p < 0.05$); different letters above bars indicate a significant difference

have been formed by chemical cleavage of DMSP. This may have been promoted by the relatively high pH [10.2 (SD 0.1)] that resulted during the experiment. At a

pH of 10.2 the first-order rate constant for chemical DMSP decomposition K is 1.94×10^{-6} s^{-1} (at 25 °C) (Dacey and Blough 1987). We measured an initial dissolved DMSP concentration of about 1.5 µM in the incubations. The calculated DMS concentration at $t = 25$ h, using this rate constant K, is 0.25 µM $[1.5 - (1.5e^{-0.007 \times 25})]$, which is close to the concentrations we measured (Fig. 3B).

In sediment containing *C. roscoffensis* DMSPp was not significantly affected by salinity, although it was somewhat lower at 19 PSU than at 32 and 40 PSU (Fig. 1B, C). DMSPp after 41.5 h of incubation was not significantly different from the DMSPp at the start of the experiment (not shown). Biomass in the incubations, expressed as Chl *a*, also did not significantly change during the experiment (not shown). After 41.5 h of
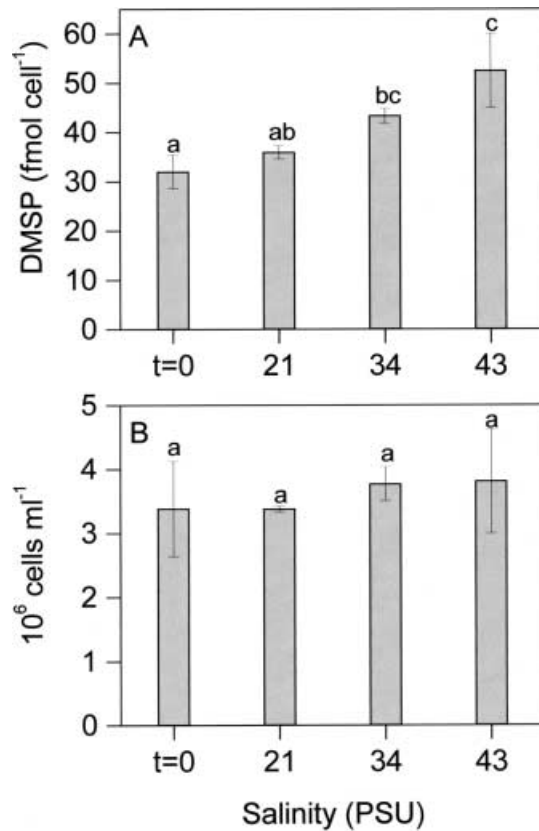
Fig. 2 DMSP per cell (**A**) and cell number (**B**) in suspensions of *Tetraselmis* sp. after incubation at different salinities for 41.5 h. Error bars: SD of mean values of three replicate incubations. Significance of differences between means tested with two-way ANOVA, followed by paired comparison (Tukey HSD-test, $p < 0.05$); different letters above bars indicate a significant difference

incubation DMSPd was somewhat higher and the DMS concentration was much higher at 19 than at 32 and 40 PSU in sediment containing *C. roscoffensis* (Fig. 1A). However, a very high amount of DMS was formed only after 24 h of incubation (Fig. 3A).

Incubation of *Tetraselmis* sp. for 2 days in medium without nitrogen or at low temperature did not have an effect on the amount of DMSP per cell (not shown).
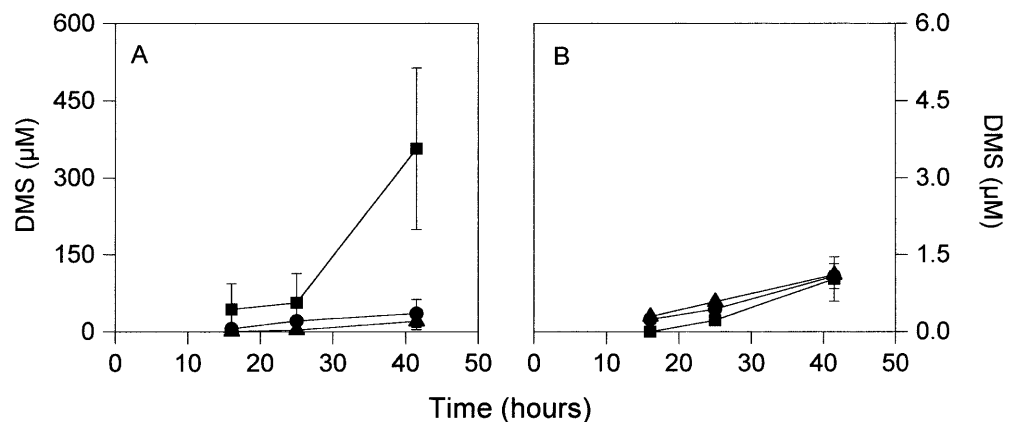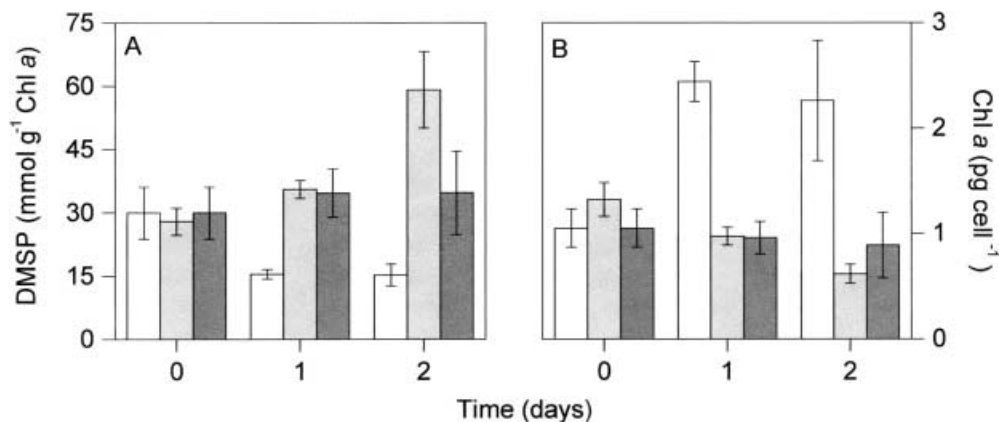
However, it did have an effect on the Chl *a* content of the cells (Fig. 4B). The amount of Chl *a* doubled in incubations in nitrogen-replete medium at 26 °C and it was halved in incubations in nitrogen-deplete medium at 26 °C. No change was observed in incubations in nitrogen-replete medium at 6 °C. These changes in Chl *a* content explain the changes in Chl *a*-specific DMSP content (Fig. 4A). It decreased in nitrogen-replete medium at 26 °C, while it increased in nitrogen-deplete medium. At 6 °C no changes occurred.

Discussion

The smell of DMS that was noticed on the beach of the intertidal mudflat of Marennes-Oléron Bay could be clearly attributed to a dense population of *Convoluta roscoffensis*. We demonstrated that this organism contained high amounts of DMSP and that its decomposition resulted in the formation of DMS. To our knowledge, the production of DMSP by animals has not been reported, so we assume that the DMSP present in *C. roscoffensis* was produced by the algal symbiont.

We have not been able to identify the factors that cause the cleavage of DMSP to DMS in this environment. We suggest that a natural turnover of *C. roscoffensis* may explain the DMS smelled in the field, since we observed that DMS was liberated when the worms died. Old sediment samples and samples fixed with glutaraldehyde, in which the worms were dying, intensely smelled of DMS. When field samples were fixed with 0.5% glutaraldehyde, a very high DMSP cleavage activity was observed. Formation of DMS in sediment after addition of glutaraldehyde was also observed by Kiene (1988) during flux experiments with salt marsh cores. Visscher et al. (1995) found that determination of DMS by headspace analysis of slurried sediment samples, fixed with 0.5% formaldehyde, yielded a concentration of DMS that was two orders of magnitude higher than by extraction of the porewater by centrifugation and subsequent purge-trapping of the DMS. Also isolated DMSP-lyases are not easily inhibited (De Souza

Fig. 3 DMS formation in vials with sediment containing *Convoluta roscoffensis* (**A**) and with suspensions of *Tetraselmis* sp. (**B**), incubated at different salinities. **A** and **B**, respectively: *squares*: 19 and 21 PSU; *circles*: 32 and 34 PSU; *triangles*: 40 and 43 PSU. Error bars: SD of mean values of four (*C. roscoffensis*) or three (*Tetraselmis* sp.) replicate incubations

and Yoch 1996; Van der Maarel et al. 1996a). But the cleavage rate we observed in our field samples still seems very high. The samples were fixed with the same buffered glutaraldehyde solution as was used by Jonkers et al. (1998) to inhibit DMSP-cleavage in sediment samples. They found that after 4 days only 6% of the initial DMSP (~900 μM) was cleaved to DMS, while in our samples all of the DMSP (final conc. ~500 μM) was cleaved within 7 h. The fixative we used was prepared in distilled water instead of seawater (Jonkers et al. 1998), which might have caused leakage of DMSP out of the cells and the mixing of DMSP with a DMSP-lyase. This still would not explain why DMSP cleavage was not inhibited. The pH of the buffered glutaraldehyde solution was 4. Therefore, the observed fast cleavage cannot have been a chemical cleavage, which was confirmed by the fact that pure DMSP (2 mM) was not cleaved when it was dissolved in glutaraldehyde. Hence, DMSP must have been cleaved enzymatically. The pH of 4 may not have been inhibitory to the DMSP-lyase, since several DMSP-lyases of micro- and macroalgae have been found to have an optimum at pH 5 or 6 and still showed substantial activity at pH 4 (Steinke et al. 1998). The isolated symbiotic alga (*Tetraselmis* sp.) did not show DMSP-lyase activity. The detection limit of our DMSP-lyase assay was about 0.1 pmol min^{-1} mg^{-1} protein, which is much lower than lyase activities reported in literature. The V_{max} of DMSP-lyase activity in crude extracts of *Phaeocystis* sp. varied between 0.35 and 1.23 μmol min^{-1} mg^{-1} protein (Stefels and Dijkhuizen 1996). Steinke et al. (1996) measured activities between 0.01 and 100 nmol min^{-1} mg^{-1} protein in marine macro- and microalgae. *Tetraselmis subcordiformis*, the only other prasinophyte that has been tested, also lacked DMSP-lyase activity (Steinke et al. 1996). Therefore, it was concluded that the formation of DMS from *C. roscoffensis*, after addition of glutaraldehyde, was catalyzed by a DMSP-lyase associated with the flatworm or with bacteria present in the sediment.

Next we present some calculations which show that it is unlikely that bacteria were responsible for the cleavage of DMSP in our samples. In samples of 0.1 g of flatworm-containing sediment in 3 ml of glutaraldehyde solution, 884 μM of DMS was formed in 0.5 h. Thus the cleavage rate was 88.4 mmol DMS min^{-1} 0.1 g^{-1} sediment. Jonkers et al. (2000) measured MPN (most probable number) counts of DMSP-utilizing bacteria of 2×10^6 cm^{-3} sediment in the top layer of an intertidal sediment. Assuming that 2.5 g of sediment equals 1 cm^3, 0.1 g of sediment contains 8×10^4 cells. Assuming that 6.6×10^6 cells contain 1 μg of protein (Jonkers et al. 2000), 8×10^4 cells contain 12.12 ng of protein. This results in a cleavage rate of 7.4 mmol DMS min^{-1} mg^{-1} protein, which is three to six orders of magnitude higher than rates reported in literature (Van der Maarel et al. 1996b; Jonkers et al. 2000). Only if the sediment or flatworms contained extremely high numbers of bacteria could such a high bacterial cleavage rate have been reached. To date DMSP-lyases of animal origin have not been reported, and it would be interesting to investigate whether the flatworm produces DMSP-lyase.

The amount of DMSP that was measured in *Tetraselmis* sp. (~30 mmol DMSP g^{-1} Chl *a*) was similar to the amount measured in *T. subcordiformis* by Gröne and Kirst (1992). The DMSP content of *C. roscoffensis* was ~200 mmol g^{-1} Chl *a*, which is about sixfold higher than that of the isolated alga. Therefore, we suggest that either the Chl *a*-specific DMSP content of the symbiotic alga inside the host was higher than that of the isolated free-living alga or that DMSP accumulated in the body tissue of the flatworm. Different incubation conditions may have led to a higher DMSP content in the symbiotic alga inside the host compared to the isolated alga. Incubations of sediment containing *C. roscoffensis* at different salinities were done in seawater at 12 °C, while the incubations of *Tetraselmis* sp. were done in nutrient-rich medium at 24 °C (Fig. 1). The DMSP content of *Tetraselmis* sp. was not affected by temperature, and it is therefore unlikely that the difference in temperature between the incubations of *C. roscoffensis* and *Tetraselmis* sp. caused the difference in DMSP content. Nitrogen limitation caused an increase in the Chl *a*-specific DMSP content of *Tetraselmis* sp. from ~30 to ~60 mmol g^{-1}. So if the symbiotic alga experienced nitrogen limitation inside the host, this may partly explain a higher Chl *a*-specific DMSP content of

C. roscoffensis. On the other hand, it is possible that the flatworm accumulated DMSP in its body tissue. The amount of DMSP that we measured in *Tetraselmis* sp. was about 50 fmol cell^{-1}. Assuming the number of symbiotic algae inside an individual of *C. roscoffensis* to be 2×10^4–7×10^4 cells (Doonan and Gooday 1982), one flatworm would contain 1–3.5 nmol DMSP. In field samples of Marennes-Oléron Bay (of seawater containing *C. roscoffensis*) we measured a total DMSP content of approximately 2 mM. These samples of 0.6 ml contained about 10–50 flatworms.

The estimated DMSP content of these worms would thus be 24–120 nmol worm^{-1}, which is at least one order of magnitude higher than the 1–3.5 nmol calculated by using the algal DMSP content that we measured. It is very unlikely that the major part of the DMSP we measured in field samples was present extracellularly, since DMSPd and DMS are usually rapidly degraded by bacteria in seawater and sediment (e.g. Kiene 1988; Kiene and Bates 1990) and DMSPd measured in seawater is always lower than DMSPp (e.g. Turner et al. 1988; Dacey et al. 1998). Therefore, we suggest that part of the DMSP we measured in *C. roscoffensis* was present in its body tissue. Hill et al. (1995) also measured a higher DMSP content in raw samples of polyps from coral reefs, containing DMSP-producing endosymbiotic dinoflagellates, compared to samples from which part of the polyp tissue had been removed. Furthermore, DMSP has been found in fish and other marine organisms feeding on DMSP-containing algae (Ackman et al. 1966; Ackman and Hingley 1968; Iida and Tokunaga 1986). Recently, Tang et al. (1999) showed that the copepod *Temora longicornis* incorporated DMSP into its body tissue when fed DMSP-rich *Tetraselmis impellucida*. These authors found that the copepods contained more DMSP at higher salinity, and they suggested an osmoregulatory function of DMSP in the copepod tissue.

Our experiments do not provide strong evidence for an osmoregulatory function of DMSP in *C. roscoffensis* since DMSPp in sediment containing *C. roscoffensis* did not significantly increase with increasing salinity after incubation at different salinities for nearly 2 days. Possibly, the conditions inside the host were unfavorable for DMSP production by the algal symbiont because the intracellular DMSP content of the isolated *Tetraselmis* sp. did increase with increasing salinity. DMSP was not excreted by *Tetraselmis* sp. in response to a decrease in salinity, which does not agree with what was found for *Tetraselmis subcordiformis* by Dickson and Kirst (1986). However, the results of the incubations of sediment containing *C. roscoffensis* indicate the release of DMSP and subsequent cleavage to DMS as a result of a decrease in salinity. The total amount of dissolved DMSP and DMS in sediment containing *C. roscoffensis* after 41.5 h of incubation at 19 PSU (412.5 μM) was much higher than at 32 (73.4 μM) and at 40 PSU (43.7 μM). Because salinity was the only factor that was varied between the incubations, this must have caused the observed difference. However, a significant effect of salinity on DMS formation was observed only after more than 24 h of incubation. Although *C. roscoffensis* in the natural environment may be exposed to larger salinity shifts than were tested in the present study, we do not expect a strong effect of salinity on the dynamics of DMSP and DMS on the time scale of a tidal cycle. Moreover, *C. roscoffensis* may be able to avoid osmotic stress actively. In the field, the worms were observed to move rapidly into the sediment when it started to rain.

Summarizing, intertidal sediments covered with dense colonies of *C. roscoffensis* contained a very high amount of DMSP. These sediments also contained a very high potential for DMSP cleavage activity, as was shown by the rapid conversion of DMSP to DMS after fixation of field samples with glutaraldehyde. Under natural conditions most DMSP was contained inside the flatworms and only a small fraction was present as DMS. However, the concentration of DMS ($\sim$55 μmol l^{-1} sediment) was very high compared to concentrations that have been measured in seawater [on the order of 10 nmol l^{-1} seawater (e.g. Turner et al. 1988; Kiene and Bates 1990)] and therefore could lead to a high local emission of DMS to the atmosphere which was confirmed by the smell of DMS produced by dense populations of *C. roscoffensis*. In the area where *C. roscoffensis* occurs, anthropogenic sources of sulfur in the atmosphere are very high compared to biogenic sources (Bates et al. 1992), and therefore the emission of DMS from *C. roscoffensis* probably does not have a large impact on the local sulfur budget or climatic forcing. Nevertheless, the symbiotic association of *C. roscoffensis* represents an interesting phenomenon, and the role of DMSP in this deserves further study.

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