Vol. 710: 15–25, 2023
https://doi.org/10.3354/meps14296

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published May 4

Distinct interactions driven by DMSP between different microalgae and the phycosphere bacterium *Sulfitobacter pseudonitzschiae* H46

Lin Yang[1], Tianyi Hu[1], Shuai Wang[1], Yanchao Zhang[1], Qian Li[1], Bin Han[1,2], Li Zheng[1,2,*]

[1]Key Laboratory of Marine Eco-Environmental Science and Technology, First Institute of Oceanography, Ministry of Natural Resources, Qingdao 266061, PR China

[2]Laboratory for Marine Ecology and Environmental Science, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao 266071, PR China

ABSTRACT: Microalgae-associated bacteria affect the dynamic succession of microalgal communities. The composition of the bacterial community of a single species of microalgae is conserved, and the same bacterial species can be found in different algal phycospheres. However, it is unknown whether the same bacterial species, widely distributed in the phycosphere, carries out the same interactions with different microalgae. Here, the interactions of *Sulfitobacter pseudonitzschiae* strain H46 and 4 microalgae, *Phaeodactylum tricornutum*, *Cylindrotheca closterium*, *Heterosigma akashiwo*, and *Chattonella marina*, were investigated using a co-cultivation method. The results showed that H46 had a significant inhibitory effect on the growth of *C. marina* but no effect on the other 3 microalgae. Further study revealed that H46 produced heat-resistant algicidal metabolites to lyse *C. marina* specifically, and secreted indoleacetic acid (IAA), which promoted the growth of the other 3 microalgae. Dimethylsulfoniopropionate (DMSP), produced by *P. tricornutum*, *C. closterium*, and *H. akashiwo*, can be utilized by H46. However, DMSP was not detected in *C. marina*, suggesting that *S. pseudonitzschiae* promotes the growth of DMSP-producing microalgae and kills DMSP-deficient microalgae. We speculate that DMSP may be a regulatory substance driving distinct algae–bacteria interactions, which is of great ecological significance for regulating the structure of microalgal communities.

KEY WORDS: *Sulfitobacter pseudonitzschiae* · Algae–bacteria interaction · Dimethylsulfoniopropionate · DMSP · Phycosphere · Indoleacetic acid · IAA

Resale or republication not permitted without written consent of the publisher

1. INTRODUCTION

Marine microalgae are the main primary producers in marine ecosystems. Despite low biomass — accounting for less than 1% of the total photosynthetic biomass on earth — marine microalgae provide more than 50% of the earth's productivity (Falkowski 1994, Field et al. 1998) and play an important role in maintaining marine ecological equilibrium.

The volume in which microalgae and bacteria interact is known as the phycosphere. Among the factors influencing the growth of microalgae, microalgae-associated bacteria, namely, phycosphere bacteria, have attracted the attention of marine ecologists (Bell & Mitchell 1972, Seymour et al. 2017). Distinct metabolic strategies of phycosphere bacteria affect their behaviors when interacting with microalgae (Buchan et al. 2014). On the one hand,

*Corresponding author: zhengli@fio.org.cn

© Inter-Research 2023 · www.int-res.com

microalgae and phycosphere bacteria can interact in a mutually beneficial relationship. The extracellular polysaccharides secreted by microalgae can be used by phycosphere bacteria as nutrients, supporting bacterial growth (Smith et al. 1995, Urbani et al. 2005, Ramanan et al. 2016). At the same time, phycosphere bacteria can synthesize and release prebiotic metabolites into the phycosphere, such as vitamin B_{12} (Croft et al. 2005) and auxin indoleacetic acid (IAA) (Amin et al. 2015, Segev et al. 2016), which can be absorbed and utilized by microalgae to promote their growth. On the other hand, some phycosphere bacteria have been found to inhibit the growth of microalgae when algal cells are in the declining phases of algal blooms or during the late stable growth period. In most cases, bacteria secrete algicidal substances to lyse microalgal cells. For example, *Ruegeria pomeroyi* produces a secondary metabolite, a lactone, to inhibit the growth of *Chlorella fusca* (Riclea et al. 2012). *Phaeobacter gallaeciensis* produces an algae-lysing substance, 'roseobacticide', causing lysis of the microalga *Emiliania huxleyi* (Seyedsayamdost et al. 2011). It has been thought that the interactions of the alga–bacterium relationship play an important role in maintaining the dynamic succession of microalgal communities (Ramanan et al. 2016).

The phycosphere is filled with metabolic substances released by microalgae, and these metabolic substances attract specific populations of bacteria that form a stable phycosphere bacterial community over a long period of coevolution (Amin et al. 2012, Mühlenbruch et al. 2018). A previous study proposed that microalgal species play a decisive role in shaping the structural composition of phycosphere bacterial communities (Kimbrel et al. 2019). Guannel et al. (2011) analyzed the phycosphere bacterial diversities of different species of the diatom genus *Pseudo-nitzschia* and found that the bacterial communities differed depending on the species and toxicity of diatoms. In contrast, it has been discovered that the structure of the phycosphere bacterial community of the same microalgae is conserved. Ramanan et al. (2015) analyzed the phycosphere bacterial diversities of 11 single-celled green algae in different habitats, including *Botryococcus braunii*, *Chlamydomonas reinhardtii*, and *Nannochloris* sp., and found that their phycosphere bacterial community compositions are similar even if they live in different environments. In addition, although the diatoms *Asterionellopsis glacialis* and *Nitzschia longissima* each have unique phycosphere bacterial communities, the bacterial community compositions of different algal strains of the same species are highly conserved at the genus level even after a long period of culture (Behringer et al. 2018). It has been speculated that microalgae and bacteria complete mutual selection through chemical exchanges and ecological interactions to form a stable phycosphere bacterial community structure (Amin et al. 2012, Ramanan et al. 2015). From the above evidence, we can conclude that the phycosphere bacteria of different algal species have different community compositions and remain stable in the same algal phycosphere.

Subsequent investigations have shown that the same genus of bacteria can appear in different algal phycospheres. *Sulfitobacter* is a genus in the Family *Roseobacteraceae* of the α-*Proteobacteria*. It was found to exist in the phycospheres of different diatoms (e.g. Schäfer et al. 2002, Kaczmarska et al. 2005, Hong et al. 2015). *Sulfitobacter* develops mutually beneficial symbioses with its host diatom by delivering IAA to the diatom and obtaining tryptophan from the diatom (Amin et al. 2015). In addition, in a previous experiment (Hu et al. 2021, Zhang et al. 2022), we isolated a *Sulfitobacter* strain, *S. pseudonitzschiae* H46, from the phycosphere of 2 microalgae: *Prorocentrum donghaiense* and *Heterosigma akashiwo*. Interestingly, *Sulfitobacter* can secrete algicidal compounds to attack *P. donghaiense* (Zhang et al. 2020). How *Sulfitobacter* interacts with *H. akashiwo*, a raphidophyte microalga, has not been studied. Despite the discovery of *Sulfitobacter* from the phycosphere of various microalgal species, there is no explanation for why the same bacterial species or group in different algal phycospheres exhibit different algae–bacteria interaction relationships or algae–bacteria symbiosis patterns.

According to previous studies, DMSP may participate in the alga–bacterium interaction. For instance, *Sulfitobacter* strain D7 was shown to inhibit *E. huxleyi*, which produces DMSP. The authors found that strain D7 can consume and metabolize DMSP to methanethiol, which promotes its growth (Barak-Gavish et al. 2018). In contrast, *Sulfitobacter* strain SA11 upregulates its DMSP lyase (*dddL*) transcripts in response to algal DMSP in the beneficial interaction with diatom *Pseudo-nitzschia multiseries* (Amin et al. 2015). The addition of DMSP can enhance the growth of *Skeletonema marinoi*, which seems to be mediated by phycosphere bacteria (Johansson et al. 2019). Previous studies found that H46 possesses the ability to inhibit the growth of *Alexandrium tamarense* and *Chattonella marina* (Hu et al. 2021) and to

degrade DMSP (Zhang et al. 2022). However, Hu et al. (2021) only described a preliminary phenomenon of microalgal inhibition by H46 without investigating whether the inhibition was related to DMSP. Zhang et al. (2022) reported that H46 can degrade DMSP but did not determine how DMSP participates in the alga–bacterium interaction.

To understand whether H46 interacts with Raphidophytes in different ways, determine the causes driving the different alga–bacterium interactions, and elucidate the possible regulating effect of DMSP in alga–bacterium interactions, we chose 2 Raphidophyceae microalgae, *H. akashiwo* and *C. marina*, and 2 model diatom strains, *Phaeodactylum tricornutum* and *Cylindrotheca closterium*, as reference strains in our study.

Co-culture experiments with the 4 microalgae species and H46 were performed to investigate the growth status of microalgae and the interaction relationship of different alga–bacterium combinations. We determined the concentration of DMSP produced by microalgae and the effect of IAA on the growth of microalgae. The factors driving different interactions between phycosphere bacterium H46 and different microalgae are discussed.

2. MATERIALS AND METHODS

2.1. Strains and culture

Zhang et al. (2022) isolated strain *Sulfitobacter pseudonitzschiae* H46 from *Heterosigma akashiwo*. It was cultured in 2216E medium (5 g peptone, 1 g yeast extract, 0.01 g $FePO_4 \cdot 4H_2O$, 1 l sterilized seawater, pH 7.0). DMSP medium (0.6 mM DMSP, 4 mM ammonium chloride, 30 nM monosodium phosphate, 100 nM ferrous chloride-ethylenediaminetetraacetic acid, 100 nM zinc chloride, 100 nM manganese(II) chloride, 1 nM cobalt(II) chloride and 1 ml l^{-1} medium of f/2 vitamin solution in 1 l sterilized seawater) was used to determine the DMSP degradation capacity of strain H46. Sodium propionate medium (1 mM sodium propionate replacing 0.6 mM DMSP in the above-described DMSP medium) was used as a control group of solo carbon resources compared with the DMSP medium (Johnson et al. 2016).

The 4 microalgae used in this study, *Phaeodactylum tricornutum*, *Cylindrotheca closterium*, *H. akashiwo*, and *Chattonella marina*, were provided by the Algae Collection of the Institute of Oceanology Chinese Academy of Sciences. Microalgae were cultured in f/2 seawater medium (Guillard & Ryther 1962). The number of algal cells was counted under a light microscope (Olympus IX51).

2.2. Analysis and identification of algae–bacteria interaction genes

Whole-genome analysis and coding gene prediction for H46 were performed by Hu et al. (2021). The DMSP degradation gene *dddL* (GenBank accession no. QYL02930.1) and the IAA synthesis-related gene encoding flavin adenine dinucleotide (FAD)-dependent oxidoreductase (no. QKS08779.1) were annotated. Phylogenetic analysis of the amino acid (aa) sequences of these 2 genes was carried out using MEGA version 6 (Tamura et al. 2013). The sequences were aligned using ClustalW. The space penalty and extended penalty for pairwise alignment were 10 and 0.1, and those for multiple alignment were 10 and 0.2. The neighbor-joining method was used to construct the phylogenetic tree with a bootstrap value of 1000.

2.3. DMSP degradation capacity of H46

To confirm that DMSP can be degraded to DMS by H46, using the method of Zhang et al. (2022), H46 (0.1 ml) in 2216E medium (optical density $[OD]_{600\ nm}$ = 1) was transferred to 1 ml of DMSP medium in a 20 ml sterilized sealed headspace vial (Agilent) in triplicate. The cells were cultured at 25°C and 200 rpm in the dark, with analyses made after 2 h and 12 h.The blank control was set as 1 ml of DMSP medium without H46. The DMS detection method was performed using a gas chromatograph (GC; Agilent 7890B) equipped with a headspace sampler (Agilent 7697A) (Williams et al. 2019).

2.4. IAA production by H46

A 1 ml aliquot of H46 in 2216E medium (OD_{600nm} = 1) was transferred to 100 ml of 2216E medium supplemented with 0.02 g L-tryptophan (Aladdin) and cultured at 30°C and 150 rpm for 2 d. The culture was centrifuged at 4°C and 10 222 × *g* for 5 min, and the supernatant was retained. The content of IAA in the supernatant was determined with Salkowski reagents at $OD_{540\ nm}$ (Bric et al. 1991, Kong 2017). An IAA standard (Solarbio) dissolved in ethanol was used for the standard curve determination, with a

concentration gradient of 0, 1×10^3, 2×10^3, 3×10^3, 4×10^3, and 5×10^3 ng ml^{-1}.

2.5. Co-culture of algae and bacteria

The 4 microalgae used in the experiment, *P. tricornutum*, *C. closterium*, *H. akashiwo*, and *C. marina*, were separately cultured in 150 ml of f/2 medium to cell densities ranging from 1.00×10^6 to 2.00×10^6 cells ml^{-1} for the first 3 species and 2.00×10^3 to 2.50×10^3 cells ml^{-1} for *C. marina*. Strain H46 was cultured in 100 ml 2216E medium to OD_{600} = 1.0 and then centrifuged at 4°C and 10 222 × *g* for 5 min. The bacteria were resuspended in 100 ml f/2 seawater medium. Then, 1.5 ml of bacterial suspension was added to 150 ml of microalgal culture liquid for co-culture, and 1.5 ml of f/2 medium was added for algal culture. Triplicate cultures were plotted at 18°C, and 72 µmol photons m^{-2} s^{-1} d^{-1} for 12 h light and 12 h dark. The microalgal cell numbers were counted over 14 d of cultivation.

2.6. DMSP consumption in an algae–bacteria co-culture system

To understand the bacterial consumption of DMSP produced by microalgae, we set up the aforementioned alga–bacterium co-culture system. Because the culture system was not sealed, the DMS produced by bacterial degradation of microalgae DMSP will volatilize. Therefore, we cannot detect DMS directly but only after addition of NaOH to the culture to hydrolyze the total DMSP (dissolved + particulate DMSP) into DMS after all the culture experiments were completed (Williams et al. 2019). On the 10th day of incubation, 1 ml of algal culture and alga–bacterium co-culture (described in Section 2.5) and 1 ml f/2 medium as a control were transferred into a 20 ml headspace vial (Agilent) in triplicate. Then, 1 ml of 10 mol l^{-1} NaOH, which hydrolyzes DMSP to DMS in alkaline conditions, was pipetted into the vial, which was then hermetically stored in the dark for 12 h (Williams et al. 2019). The DMS detection method by GC is consistent with the method described in Section 2.3. The DMSP concentration in the sample is reflected in the content of equimolar DMS. The DMSP concentration in algal culture minus the DMSP concentration in co-culture is equal to the DMSP consumption by H46. The bacterial consumption rate of DMSP was calculated according to the equation:

$$\text{DMSP Consumption Rate} = \frac{C_{\text{DMSP.Control}} - C_{\text{DMSP.Coculture}}}{C_{\text{DMSP.Control}}} \times 100\% \qquad (1)$$

where $C_{\text{DMSP.Control}}$ is the DMSP concentration in algal culture and $C_{\text{DMSP.Coculture}}$ is the DMSP concentration in co-culture (algae + bacterium).

2.7. Effect of IAA on the growth of microalgae

The 4 microalgae, *P. tricornutum*, *C. closterium*, *H. akashiwo*, and *C. marina*, were separately cultured in f/2 medium to cell densities ranging from 0.50×10^6 to 1.00×10^6 cells ml^{-1} for the first 3 species and 1.50×10^3 to 2.00×10^3 cells ml^{-1} for *C. marina*. IAA (Solarbio) was added to the microalgal culture liquid to a final concentration of 300 ng ml^{-1}. At the same time, f/2 medium with IAA concentration gradients of 0, 10^{-2}, 10^{-1}, 1, 10, and 100 ng ml^{-1} was used for *C. marina* cultivation. Cultivations were performed in triplicate at 18°C, 72 µmol photons m^{-2} s^{-1} d^{-1} under 12 h light and 12 h dark conditions. The microalgal cell numbers were counted over 14 d of cultivation.

2.8. Algicidal mode of strain H46

H46 was cultured in 150 ml 2216E medium at 30°C and 150 rpm for 24 h. Then, the bacteria were centrifuged at 4°C and 10 222 × *g* for 5 min, and the supernatant was filtered through a 0.22 µm filter membrane. Half of the supernatant was heated at 95°C for 30 min as a heat treatment supernatant sample. Another portion of the supernatant was untreated. For the bacteria, 150 ml of sterilized seawater was added for resuspension.

During the logarithmic growth phase, 0.5 ml of *C. marina* was transferred to 4.5 ml of fresh f/2 medium. Then, 0.05 ml of resuspended H46 bacterium, untreated supernatant, and heat-treated supernatant were added to the algal culture separately and in triplicate. Simultaneously, 0.05 ml of f/2 medium was added as a blank control. The number of *C. marina* cells was counted after 0.5 and 12 h of cultivation, and the morphology of *C. marina* was observed with an ECLIPSE TE2000-U fluorescence inverted microscope (Nikon).

2.9. Data analysis

The difference in DMS concentration between the H46 group and the blank group, the differ-

ence in DMSP concentration between the coculture and algal culture, and the difference in algal cell counts between the treatment group and the control group were analyzed using the Shapiro-Wilk test and the D'Agostino & Pearson test for normality. These unpaired parametric tests assume that the data are normally distributed. After variances were compared by an *F*-test, an unpaired *t*-test or unpaired *t*-test with Welch's correction was used to test for significant differences, with a significance level (α) of 0.05. All data measured in the experiments were plotted as lines or histograms, and significant differences were analyzed using GraphPad Prism version 8.0.2 (GraphPad Software).

3. RESULTS

3.1. Annotation and functional identification of algae–bacteria interaction-related genes

Based on the whole-genome analysis of H46 by Hu et al. (2021), we confirmed 2 algae–bacteria interaction-related protein sequences: DMSP lyase DddL (QYL02930.1, 253 aa) and IAA synthesis-related protein FAD-dependent oxidoreductase (QKS08779.1, 557 aa). DMSP and IAA have been recognized as playing an important role in the algae–bacteria symbiotic relationship (Amin et al. 2015, Töpel et al. 2019). By phylogenetic analysis (Fig. 1), the DMSP lyase DddL in H46 was found to be closely related to

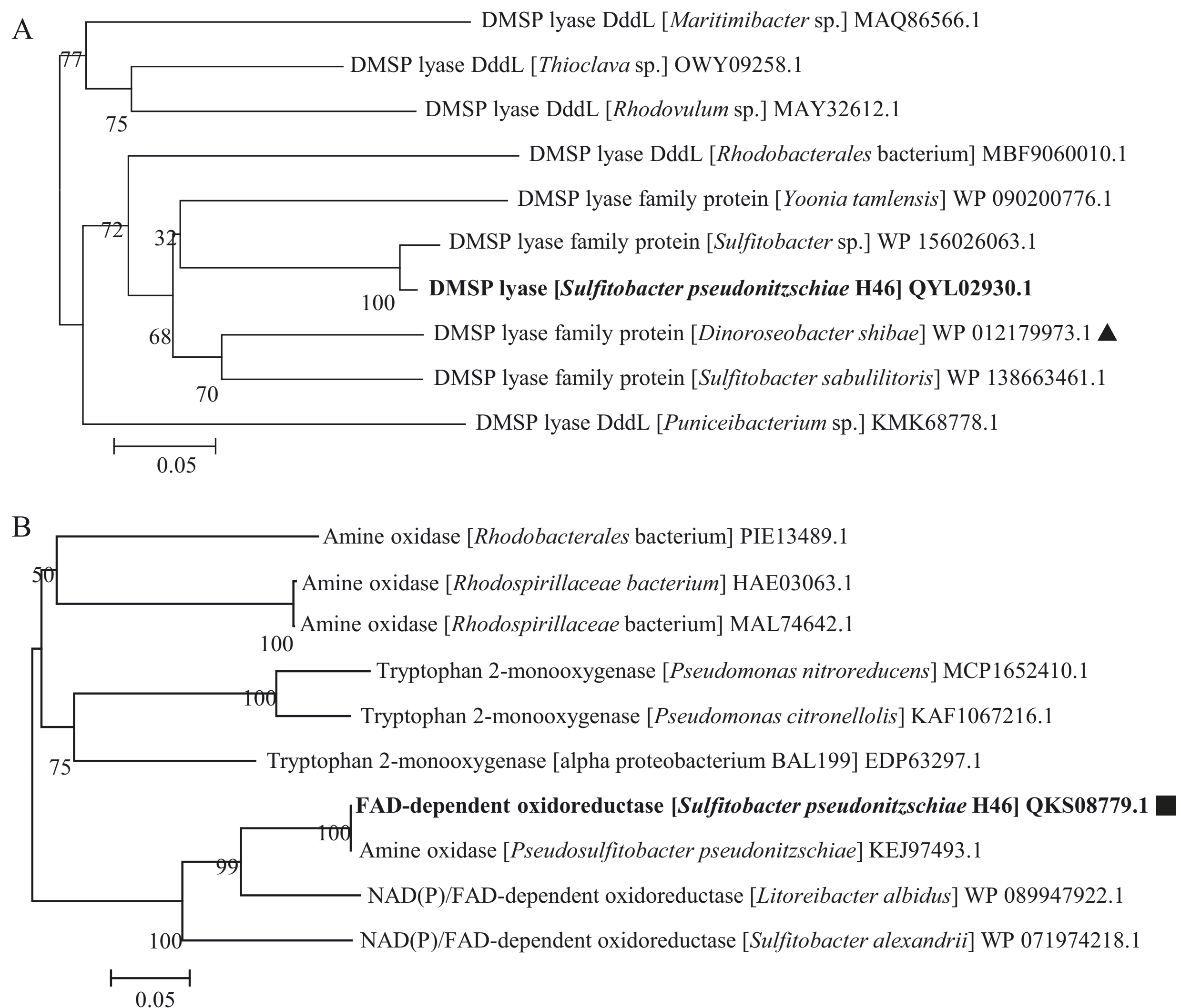

Fig. 1. Phylogenetic tree of (A) the DMSP lyase DddL (▲) and (B) flavin adenine dinucleotide (FAD)-dependent oxidoreductase (■) constructed by complete protein sequences (253 aa in DddL and 557 aa in FAD-dependent oxidoreductase) of the phycosphere bacterium *Sulfitobacter pseudonitzschiae* H46. Phylogenetic trees were constructed by the neighbor-joining method, with a bootstrap value of 1000

the DMSP lyase family protein (WP 156026063.1) in *Sulfitobacter* sp., with a similarity of 97.02%. The FAD-dependent oxidoreductase in H46 was closely related to the amine oxidase (KEJ97493.1) in *Pseudosulfitobacter pseudonitzschiae*, with a similarity of 99.82%.

The ability of H46 to degrade DMSP into DMS was determined using GC. The results show that the presence of H46 significantly increased the degradation of DMSP (unpaired *t*-test with Welch's correction for 2 h: Welch-corrected $t = 10.1$, df = 2, $p = 0.005$; unpaired *t*-test for 12 h: $t = 321$, df = 4, $p < 0.001$) (Fig. 2). In the blank group without H46, DMS was barely detected. In contrast, the H46 groups produced noticeable DMS. The amount of DMS increased with time. The DMS concentrations at both 2 and 12 h were higher than 10 µmol l^{-1} (10.97 and 28.73 µmol l^{-1}, respectively).

Subsequently, we tested the capacity of H46 to produce IAA. After cultivation in 2216E medium supplied with L-tryptophan for 2 d, IAA was detected in the supernatant using Salkowski reagents. Combined with the standard curve of IAA measured at $OD_{540\ nm}$, the concentration of IAA in the H46 supernatant was calculated to be 279 ng ml^{-1}. These results indicate that H46 has the ability to degrade DMSP and synthesize IAA.

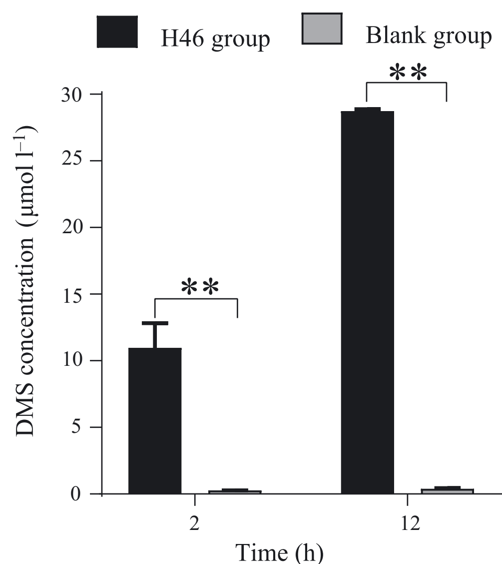

Fig. 2. Ability of *Sulfitobacter pseudonitzschiae* H46 to degrade DMSP into DMS 2 and 12 h after cultivation. Detection was performed by gas chromatography on DMSP medium with (H46 group) and without (blank group) H46. Asterisks indicate significant differences (**$p < 0.01$) between groups at each detection time. Error bars indicate SD

3.2. Growth effect of microalgae in an algae–bacteria co-culture system

H46 exhibited a distinct symbiotic relationship with different microalgae. As described in Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m710p015_supp.pdf, microalgae showed logistic growth in algal culture. However, when co-cultured with H46, the growth of *C. marina* was inhibited significantly (unpaired *t*-test with Welch's correction: Welch-corrected $t = 2.72$, df = 7.24, $p = 0.015$), while the algal cell counts of the other 3 microalgae continued to increase. Thus, H46 specifically inhibited *C. marina*.

3.3. DMSP production by microalgae and consumption by H46

H46 has the ability to utilize DMSP produced by microalgae. DMSP was detected in the algal cultures of *P. tricornutum*, *C. closterium*, and *H. akashiwo*, and the total DMSP concentration reached 0.68, 0.85, and 1.89 µmol l^{-1}, respectively, after 10 d of cultivation. Additionally, it is worth noting that this is the first report of DMSP in *H. akashiwo*, a raphidophyte. However, DMSP was not detected in the algal culture medium or in the co-culture of *C. marina* and the negative control f/2 medium. In the co-culture system of the 3 DMSP-producing microalgae, DMSP was observed to be degraded by H46 (*P. tricornutum*, unpaired *t*-test: $t = 38.93$, df = 4, $p < 0.001$; *C. closterium*, unpaired *t*-test: $t = 7.639$, df = 4, $p < 0.001$; *H. akashiwo*, unpaired *t*-test: $t = 25.95$, df = 14, $p < 0.001$). The DMSP concentration in the *P. tricornutum*, *C. closterium*, and *H. akashiwo* co-culture systems was reduced to 0.12, 0.54, and 0.95 µmol l^{-1}, respectively, showing that the rate of DMSP consumption by H46 was 83.00, 36.33, and 49.67%, respectively (Fig. 3).

3.4. Growth effect of IAA on microalgae

The growth status of the 4 microalgae in the IAA environment was similar to that in the H46 co-culture environment. As shown in Fig. S2, all algal cultures of the 4 microalgae exhibited logistic growth. When cultivated in the IAA environment, *C. marina* showed a sharp algal cell-count decline, and the number of cells approached 0 cells ml^{-1} on Day 4, showing significant inhibition (unpaired *t*-test with

Welch's correction: Welch-corrected t = 4.85, df = 8.89, $p < 0.001$). The other 3 microalgae did not show growth inhibition in the IAA environment.

Furthermore, we found that *C. marina* could grow in the presence of a low concentration of IAA but was inhibited in the presence of a high concentration of IAA, as indicated in Fig. 4. The number of cells on Day 10 was significantly higher compared with the initial conditions in the IAA series at a concentration of 0 ng ml^{-1} (unpaired t-test: t = 4.439, df = 4, p = 0.011), 10^{-2} ng ml^{-1} (unpaired t-test: t = 3.498, df = 4, p = 0.025), 10^{-1} ng ml^{-1} (unpaired t-test with Welch's correction: Welch-corrected t = 16.33, df = 3.268, $p < 0.001$), and 1 ng ml^{-1} (unpaired t-test: t = 5.264, df = 4, p = 0.006), but decreased significantly to 0 cells ml^{-1} at concentrations of 10 ng ml^{-1} (unpaired t-test with Welch's correction: Welch-corrected t = 7.173, df = 2, p = 0.019) and 100 ng ml^{-1} (unpaired t-test with Welch's correction: Welch-corrected t = 17.34, df = 2, p = 0.003). These results indicate that high concentrations of IAA inhibit the growth of *C. marina*.

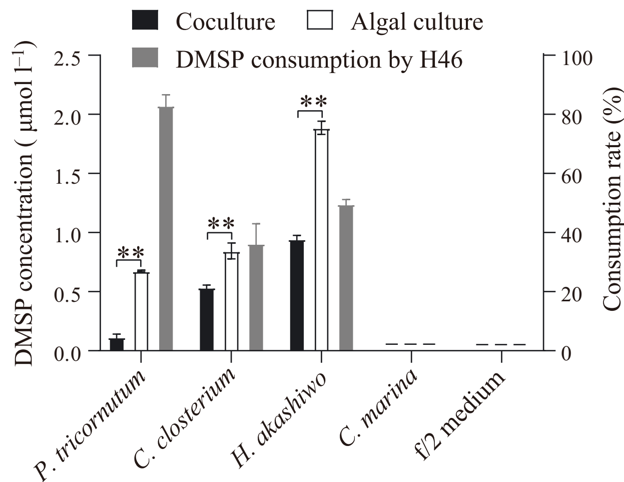

Fig. 3. DMSP concentration in the culture system and rate of DMSP consumption by H46. H46 was present in the coculture system but not in the algal culture system. The negative control is f/2 medium. Detection was performed on the tenth day after cultivation. Asterisks indicate significant differences (**$p < 0.01$) between algal culture and coculture groups. Error bars indicate SD

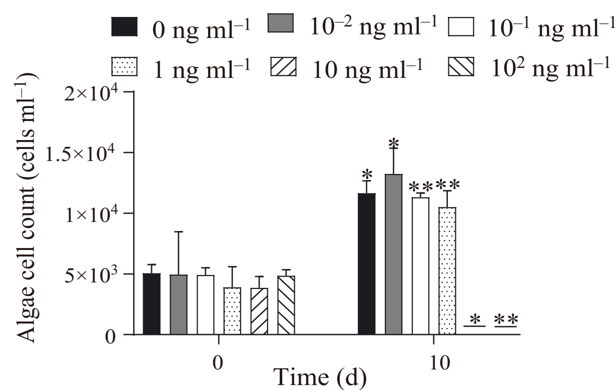

Fig. 4. Growth effect of *Chattonella marina* when cultured in f/2 medium with different concentrations of indoleacetic acid (IAA). Cell counts at each IAA concentration were conducted on Days 0 and 10 after incubation. Asterisks indicate significant differences (*$p < 0.05$; **$p < 0.01$) in cell numbers between Days 0 and 10 for each group. Error bars indicate SD

3.5. Algicidal mode of H46 against *C. marina*

The supernatant of H46, with or without heat treatment, enabled the lysis of *C. marina*, causing a decrease in counted cells (supernatant untreated group, unpaired t-test: t = 9.385, df = 4, $p < 0.001$; supernatant heat treatment group, unpaired t-test: t = 19.48, df = 4, $p < 0.001$) (Fig. 5). When co-cultured with H46 cells, the number of *C. marina* cells was maintained at the initial level with no obvious change (unpaired t-test: t = 2.345, df = 4, p = 0.079), consistent with the results of the algae–bacteria coculture experiment (Fig. S1). The number of *C. marina* cells in the algal culture increased (unpaired t-test: t = 2.025, df = 4, p = 0.113).

In terms of cell morphology, cell debris was observed when *C. marina* was co-cultured with the H46 supernatant and H46. As illustrated in Fig. 6B,

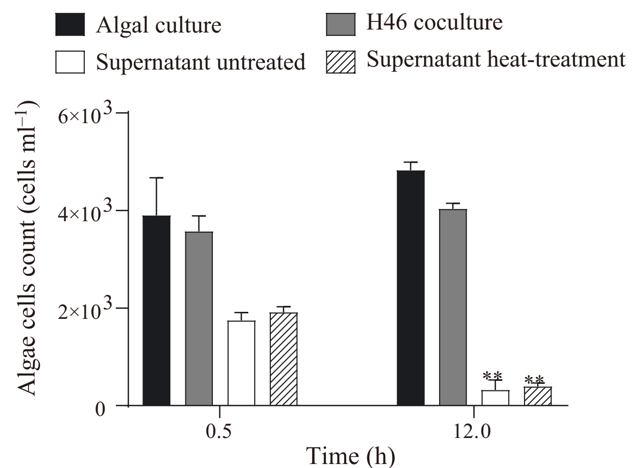

Fig. 5. Algicidal effect of H46 on *Chattonella marina*, cultured in f/2 medium with H46 cells or supernatant added. Algae cells were counted 0.5 and 12 h after incubation. Asterisks indicate significant differences (**$p < 0.01$) in cell numbers between groups. Error bars indicate SD

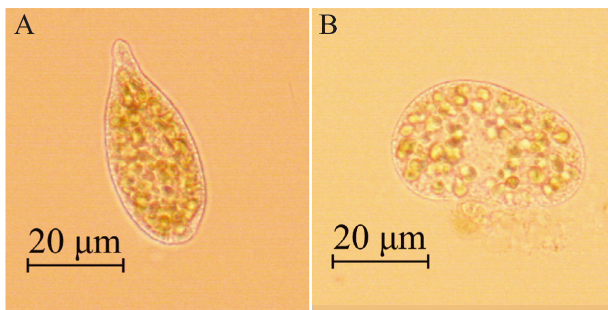

Fig. 6. Cell morphology of *Chattonella marina* in (A) algal culture and (B) H46 co-culture

swollen cells and lysed membranes appeared, accompanied by blurry cell boundaries and cell content release. However, it is worth noting that the number of intact cells in the co-culture group (4.03×10^3 cells ml^{-1}) was greater than that in the supernatant group (320 cells ml^{-1}).

These results reveal that the algicidal activity of H46 against *C. marina* was not accomplished through direct contact with microalgae. The bacteria produce thermostable algicidal metabolites that cause algal lysis.

4. DISCUSSION

Sulfitobacter is a widespread genus existing in different phycospheres (e.g. Grossart et al. 2005, Sapp et al. 2007, Töpel et al. 2019); whether it remains symbiotic with various microalgae is still unknown. In this study, one phycosphere strain, *Sulfitobacter pseudonitzschiae* H46, was co-cultivated with 4 microalgal strains, and their interactions were investigated. Our results show that H46 exhibits a distinct symbiotic pattern with different microalgae. Specifically, H46 did not show an inhibitory effect on the growth of *Phaeodactylum tricornutum*, *Cylindrotheca closterium*, and *Heterosigma akashiwo* but significantly inhibited the growth of *Chattonella marina* and lysed its cells.

The symbiotic relationships of *Sulfitobacter* and diatoms have been well studied. In a previous study, *Sulfitobacter* SA11 was found to utilize tryptophan produced by the diatom *Pseudo-nitzschia multiseries* to synthesize and secrete IAA into the phycosphere, which was absorbed by diatoms and promoted their growth (Amin et al. 2015). Our results are consistent with this previous study, showing that the diatoms *P. tricornutum* and *C. closterium* can grow logistically in the presence of *Sulfitobacter*. Intriguingly, IAA was also detected in the metabolites of H46. Further study showed that IAA did not inhibit the growth of diatoms and *H. akashiwo*. This result indicates that bacterial strain H46 is harmless to these 3 microalgae.

C. marina, unlike the other 3 microalgae, suffered significant inhibition when co-cultured with H46. We found that high concentrations of IAA had harmful effects on *C. marina*, but low concentrations of IAA were harmless. A similar phenomenon was observed for *Chlorella* sp. (Deng et al. 2013), whose growth was inhibited at 20 µmol l^{-1} (=3743.8 ng ml^{-1}) IAA but promoted when the IAA concentration was lower than 20 µmol l^{-1}. In our study, *C. marina* could not proliferate when the IAA concentration was higher than 10 ng ml^{-1}, while the other 3 microalgae tolerated IAA up to 300 ng ml^{-1}. Although H46 is capable of synthesizing IAA up to 279 ng ml^{-1} in the medium, the growth of *C. marina* was completely inhibited at 300 ng ml^{-1} IAA. The IAA concentration should be very low in a natural phycosphere. Considering that the promotion of microalgal growth by IAA is dose-dependent, we do not regard it as an algicidal substance of H46 against *C. marina*. According to our results, the algicidal substance produced by H46 is unlikely to be a protein due to its thermostability. As seen in Fig. 5, we note that the mechanism of microalgal killing is by lysing the cell membranes of *C. marina* with algicidal substances. This algicidal mechanism is similar to that of the algicidal bacterium *Sulfitobacter porphyrae* ZFX1 (Zhang et al. 2020). We speculate that the algicidal mechanism that damages cell membranes is accomplished by excessive production of reactive oxygen species (ROS) induced by algicidal substances, which initiate lipid peroxidation, resulting in oxidative damage to membrane systems similar to the mechanism of ZXF1 and other algicidal substances (e.g. Yang et al. 2017, Zhang et al. 2020). Further work to identify the algicidal substance and illuminate the algicidal mechanisms of H46 is ongoing.

In a previous study of the algicidal driving factor of *Sulfitobacter* D7 against *Emiliania huxleyi*, it was proposed that secretion of algicidal substances by D7 is induced by DMSP produced by microalgae (Barak-Gavish et al. 2018). However, DMSP seemed to have a protective effect on algal cells against the algicidal bacterium H46 in our study. Our results showed that the total DMSP could be determined in the algal culture of 3 microalgae — *P. tricornutum*, *C. closterium*, and *H. akashiwo* — and the DMSP produced by different microalgae could be degraded by H46. Interestingly, these 3 microalgal species were not lysed by

H46 in the co-culture system. However, the DMSP-deficient microalga *C. marina* was susceptible to attack by H46. DMSP can facilitate microalgal survival under environmental stress. For example, when faced with osmotic and low-temperature stress, microalgae regulate intracellular osmotic pressure and stabilize the structure of their intracellular proteins, such as phosphofructokinase, lactate dehydrogenase, and glutamate dehydrogenase, by accumulating DMSP (Nishiguchi & Somero 1992, Yoch 2002, Gebser & Pohnert 2013). Moreover, DMSP plays a role as an antioxidant that can protect microalgae from oxidative damage by scavenging excessive ROS, such as hydroxyl radicals, produced in microalgae under environmental stress (Sunda et al. 2002, 2005). Hence, it can be speculated that producing DMSP may be one mechanism of protection for microalgae avoiding membrane-system oxidative damage caused by algicidal substances.

In addition, the degradation products of DMSP also play important ecological roles in the phycosphere. Acrylate, one of the DMSP hydrolysates degraded by the DMSP lyase DddL, can protect the bacterium *Puniceibacterium antarcticum* SM1211 from predation by the marine ciliate *Uronema marinum* (Teng et al. 2021). We note that H46 and SM1211 both contain the *dddL* gene and cannot grow in DMSP medium (Fig. S3). Therefore, we speculate that DddL in the phycosphere bacteria H46 and SM1211 may contribute to the same ecological role, with DddL hydrolyzing DMSP and producing acrylate to induce grazing defense rather than contributing to the utilization of nutrients (Wolfe et al. 1997, Teng et al. 2021). Another degradation product of DMSP, DMS, acts as a foraging cue for both herbivorous micrograzers and top predators, which means that DMS may act as a guard for microalgae defending against zooplankton by means of trophic cascades, such as in the relationship between *Oxyrrhis marina* and *E. huxleyi* (Owen et al. 2021, Shemi et al. 2021).

Although DMSP was identified as an algicidal trigger in the phycosphere (Barak-Gavish et al. 2018), our study showed that the algicidal activity of strain H46 was not observed in microalgal cultures that contained DMSP. In contrast, the microalgal culture that did not contain DMSP suffered from the algicidal activity of H46. This finding suggests that DMSP can play different roles in alga–bacterium interactions. Here, we propose a hypothesis depicting the ecological role of algae–bacteria interactions in our study (Fig. 7). In detail, DMSP plays a role as a safeguard for its producer, defending against attack by algicidal substances. DMSP degradation products, DMS or acrylate, protect bacteria and microalgae from pred-

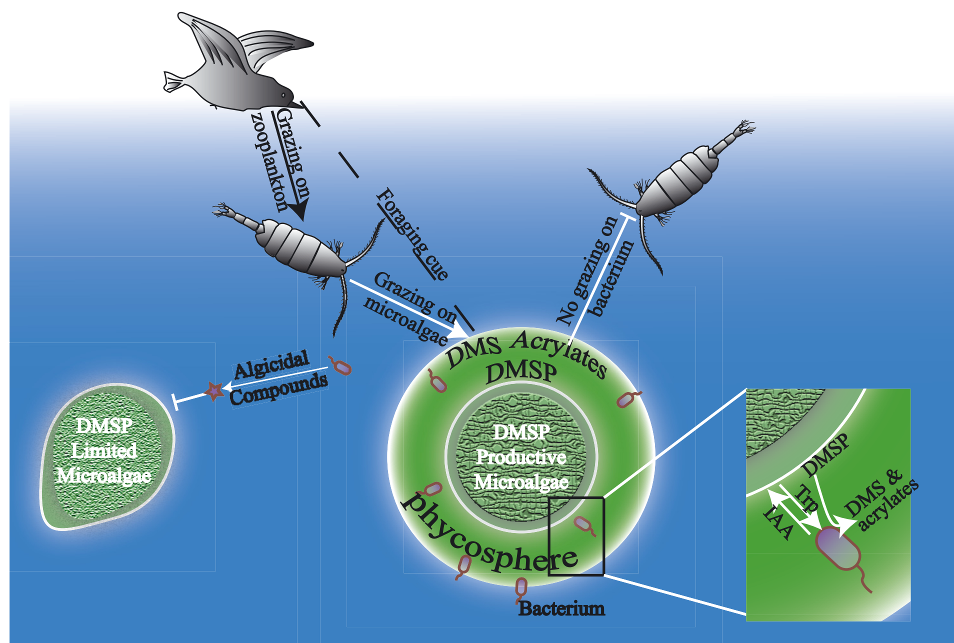

Fig. 7. Ecological relationships between the phycosphere bacterium *Sulfitobacter pseudonitzschiae* H46 and microalgae. Bacteria and DMSP-abundant microalgae form a mutualistic relationship via the exchange of DMSP and indoleacetic acid (IAA). DMSP protects the microalgae from attack by algicidal substances. DMSP-deficient microalgae suffer lysis caused by algicidal substances due to a lack of DMSP protection. DMSP hydrolysates also offer microalgae and bacteria protection from predators

ators by means of chemoresistance and trophic cascades. To obtain more DMSP, some phycosphere bacteria secrete IAA to promote the growth of DMSP-abundant microalgae. For DMSP-deficient microalgae, algicidal bacteria lyse their cells to promote DMSP-abundant microalgae to succeed in their survival niches. Consistent with our hypothesis, there is a report that *Sulfitobacter* promotes the growth of the DMSP-producing microalga *P. multiseries* (Amin et al. 2015). However, DMSP-producing microalgae can be inhibited by *Sulfitobacter*, such as *E. huxleyi* and *Alexandrium tamarense* (Barak-Gavish et al. 2018, Zhu et al. 2018, Hu et al. 2021). The content of DMSP produced by different strains of microalgae is different, even for the same species (Keller et al. 1989, Yang et al. 2022). More microalgal strains should be studied to verify our hypothesis.

In this study, we report for the first time that DMSP can be produced by *H. akashiwo*, a raphidophyte. We propose that *Sulfitobacter* H46 possesses different interaction relationships with different microalgae. DMSP produced by microalgae may act as an important regulatory factor, triggering different alga–bacterium interactions. Our results deepen the understanding of the mechanisms of algae–bacteria interactions and of phytoplankton community succession.

Acknowledgements. This work was supported by the Basic Scientific Fund for National Public Research Institutes of China (2021Q10), the National Natural Science Foundation of China (42276140 and 41776176), the National Natural Science Foundation of China–Shandong Joint Funded Project (U1606404), the Open Fund of Key Laboratory of Biotechnology and Bioresources Utilization (Dalian Minzu University), and the Ministry of Education (No. KF202006), China. We are grateful to the Algae Collection of the Institute of Oceanology Chinese Academy of Sciences for providing the microalgae.

LITERATURE CITED

Amin SA, Parker MS, Armbrust EV (2012) Interactions between diatoms and bacteria. Microbiol Mol Biol Rev 76:667–684

Amin SA, Hmelo LR, van Tol HM, Durham BP and others (2015) Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. Nature 522: 98–101

Barak-Gavish N, Frada MJ, Ku C, Lee PA and others (2018) Bacterial virulence against an oceanic bloom-forming phytoplankter is mediated by algal DMSP. Sci Adv 4: eaau5716

Behringer G, Ochsenkuhn MA, Fei C, Fanning J, Koester JA, Amin SA (2018) Bacterial communities of diatoms display strong conservation across strains and time. Front Microbiol 9:659

Bell W, Mitchell R (1972) Chemotactic and growth responses of marine bacteria to algal extracellular products. Biol Bull (Woods Hole) 143:265–277

Bric JM, Bostock RM, Silverstone SE (1991) Rapid *in situ* assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol 57:535–538

Buchan A, LeCleir GR, Gulvik CA, González JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nat Rev Microbiol 12: 686–698

Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG (2005) Algae acquire vitamin B_{12} through a symbiotic relationship with bacteria. Nature 438:90–93

Deng X, Wu X, Fan X, Fei X, Ren D (2013) Effects of indole-3-acetic acid and abscisic acid on growth and lipid accumulation of *Chlorella* sp. Zhongguo Youliao Zuowu Xuebao 35:58–63

Falkowski PG (1994) The role of phytoplankton photosynthesis in global biogeochemical cycles. Photosynth Res 39:235–258

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237–240

Gebser B, Pohnert G (2013) Synchronized regulation of different zwitterionic metabolites in the osmoadaption of phytoplankton. Mar Drugs 11:2168–2182

Grossart HP, Levold F, Allgaier M, Simon M, Brinkhoff T (2005) Marine diatom species harbour distinct bacterial communities. Environ Microbiol 7:860–873

Guannel ML, Horner-Devine MC, Rocap G (2011) Bacterial community composition differs with species and toxigenicity of the diatom *Pseudo-nitzschia.* Aquat Microb Ecol 64:117–133

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. Can J Microbiol 8:229–239

Hong Z, Lai Q, Luo Q, Jiang S, Zhu R, Liang J, Gao Y (2015) *Sulfitobacter pseudonitzschiae* sp. nov., isolated from the toxic marine diatom *Pseudo-nitzschia multiseries.* Int J Syst Evol Microbiol 65:95–100

Hu T, Wang S, Shan Y, Zhang Y, Zhu Y, Zheng L (2021) Complete genome of marine microalgae associated algicidal bacterium *Sulfitobacter pseudonitzschiae* H46 with quorum sensing system. Curr Microbiol 78:3741–3750

Johansson ON, Pinder MIM, Ohlsson F, Egardt J, Töpel M, Clarke AK (2019) Friends with benefits: exploring the phycosphere of the marine diatom *Skeletonema marinoi.* Front Microbiol 10:1828

Johnson WM, Kido Soule MC, Kujawinski EB (2016) Evidence for quorum sensing and differential metabolite production by a marine bacterium in response to DMSP. ISME J 10:2304–2316

Kaczmarska I, Ehrman JM, Bates SS, Green DH, Léger C, Harris J (2005) Diversity and distribution of epibiotic bacteria on *Pseudo-nitzschia multiseries* (Bacillariophyceae) in culture, and comparison with those on diatoms in native seawater. Harmful Algae 4:725–741

Keller MD, Bellows WK, Guillard RRL (1989) Dimethyl sulfide production in marine phytoplankton. In: Saltzman ES, Cooper WJ (eds) Biogenic sulfur in the environment. ACS Symp Ser, Vol 393. American Chemical Society, Washington, DC, p 167–182

Kimbrel JA, Samo TJ, Ward C, Nilson D and others (2019) Host selection and stochastic effects influence bacterial

community assembly on the microalgal phycosphere. Algal Res 40:101489

Kong L (2017) Screening, identification and optimization of bacterium and microalgae for high yield of auxin. MSc dissertation, China University of Petroleum, Beijing

Mühlenbruch M, Grossart HP, Eigemann F, Voss M (2018) Mini-review: phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. Environ Microbiol 20:2671–2685

Nishiguchi MK, Somero GN (1992) Temperature- and concentration-dependence of compatibility of the organic osmolyte β-dimethylsulfoniopropionate. Cryobiology 29: 118–124

Owen K, Saeki K, Warren JD, Bocconcelli A and others (2021) Natural dimethyl sulfide gradients would lead marine predators to higher prey biomass. Commun Biol 4:149

Ramanan R, Kang Z, Kim BH, Cho DH, Jin L, Oh HM, Kim HS (2015) Phycosphere bacterial diversity in green algae reveals an apparent similarity across habitats. Algal Res 8:140–144

Ramanan R, Kim BH, Cho DH, Oh HM, Kim HS (2016) Algae–bacteria interactions: evolution, ecology and emerging applications. Biotechnol Adv 34:14–29

Riclea R, Gleitzmann J, Bruns H, Junker C, Schulz B, Dickschat JS (2012) Algicidal lactones from the marine *Roseobacter* clade bacterium *Ruegeria pomeroyi.* Beilstein J Org Chem 8:941–950

Sapp M, Schwaderer AS, Wiltshire KH, Hoppe HG, Gerdts G, Wichels A (2007) Species-specific bacterial communities in the phycosphere of microalgae? Microb Ecol 53: 683–699

Schäfer H, Abbas B, Witte H, Muyzer G (2002) Genetic diversity of 'satellite' bacteria present in cultures of marine diatoms. FEMS Microbiol Ecol 42:25–35

Segev E, Wyche TP, Kim KH, Petersen J and others (2016) Dynamic metabolic exchange governs a marine algal–bacterial interaction. eLife 5:e17473

Seyedsayamdost MR, Carr G, Kolter R, Clardy J (2011) Roseobacticides: small molecule modulators of an algal–bacterial symbiosis. J Am Chem Soc 133:18343–18349

Seymour JR, Amin SA, Raina JB, Stocker R (2017) Zooming in on the phycosphere: the ecological interface for phytoplankton–bacteria relationships. Nat Microbiol 2:17065

Shemi A, Alcolombri U, Schatz D, Farstey V and others (2021) Dimethyl sulfide mediates microbial predator–prey interactions between zooplankton and algae in the ocean. Nat Microbiol 6:1357–1366

Smith DC, Steward GF, Long RA, Azam F (1995) Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. Deep Sea Res II 42:75–97

Sunda W, Kieber DJ, Kiene RP, Huntsman S (2002) An antioxidant function for DMSP and DMS in marine algae. Nature 418:317–320

Sunda WG, Litaker RW, Hardison DR, Tester PA (2005) Dimethylsulfoniopropionate (DMSP) and its relation to algal pigments in diverse waters of the Belize coastal lagoon and barrier reef system. Mar Ecol Prog Ser 287: 11–22

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725–2729

Teng ZJ, Wang P, Chen XL, Guillonneau R and others (2021) Acrylate protects a marine bacterium from grazing by a ciliate predator. Nat Microbiol 6:1351–1356

Töpel M, Pinder MIM, Johansson ON, Kourtchenko O, Clarke AK, Godhe A (2019) Complete genome sequence of novel *Sulfitobacter pseudonitzschiae* strain SMR1, isolated from a culture of the marine diatom *Skeletonema marinoi.* J Genomics 7:7–10

Urbani R, Magaletti E, Sist P, Cicero AM (2005) Extracellular carbohydrates released by the marine diatoms *Cylindrotheca closterium*, *Thalassiosira pseudonana* and *Skeletonema costatum*: effect of P-depletion and growth status. Sci Total Environ 353:300–306

Williams BT, Cowles K, Bermejo Martinez A, Curson ARJ and others (2019) Bacteria are important dimethylsulfoniopropionate producers in coastal sediments. Nat Microbiol 4:1815–1825

Wolfe G, Steinke M, Kirst G (1997) Grazing-activated chemical defence in a unicellular marine alga. Nature 387: 894–897

Yang K, Chen Q, Zhang D, Zhang H and others (2017) The algicidal mechanism of prodigiosin from *Hahella* sp. KA22 against *Microcystis aeruginosa.* Sci Rep 7:7750

Yang QQ, Li PF, Duan SS, Han L, Gao PP, Liu CY, Yang GP (2022) Production of dimethylsulfoniopropionate, dimethylsulfide and acrylic acid from marine microalgae. J Sea Res 190:102299

Yoch DC (2002) Dimethylsulfoniopropionate: its sources, role in the marine food web, and biological degradation to dimethylsulfide. Appl Environ Microbiol 68: 5804–5815

Zhang F, Fan Y, Zhang D, Chen S and others (2020) Effect and mechanism of the algicidal bacterium *Sulfitobacter porphyrae* ZFX1 on the mitigation of harmful algal blooms caused by *Prorocentrum donghaiense.* Environ Pollut 263:114475

Zhang Y, Zheng L, Wang S, Zhao Y, Xu X, Han B, Hu T (2022) Quorum sensing bacteria in the phycosphere of HAB microalgae and their ecological functions related to cross-kingdom interactions. Int J Environ Res Public Health 19:163

Zhu R, Zhang H, Zhang J, Yang G (2018) Experimental study on the production of dimethylsulfide and dimethylsulfoniopropionate in two microalgae under different conditions. Period Ocean Univ China 48:62–70

Editorial responsibility: Antonio Bode, A Coruña, Spain
Reviewed by: R. Simó and 2 anonymous referees

Submitted: October 20, 2022
Accepted: March 21, 2023
Proofs received from author(s): April 24, 2023