Title: Acrylate protects a marine bacterium from grazing by a ciliate predator

Authors: Zhao-Jie Teng[1,2,3]†, Peng Wang[1,3]†, Xiu-Lan Chen[2,3]†, Richard Guillonneau[4], Chun-Yang Li[1,3], Song-Bao Zou[5], Jun Gong[6], Kai-Wen Xu[7], Lin Han[8], Chao Wang[8], David J Scanlan[4], Yin Chen[4,1], Yu-Zhong Zhang[1,2,3]*

Affiliations:

[1]College of Marine Life Sciences, and Frontiers Science Center for Deep Ocean Multispheres and Earth System, Ocean University of China, Qingdao, China.

[2]State Key Laboratory of Microbial Technology, Marine Biotechnology Research Center, Shandong University, Qingdao, China.

[3]Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology, Qingdao, China.

[4]School of Life Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom.

[5]Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China.

[6]School of Marine Sciences, Sun Yat-Sen University, Zhuhai Campus, Tangjiawan, Zhuhai, China.

[7]Department of Electrical Engineering and Computer Science, Vanderbilt University, Nashville, TN 37235, USA.

[8]Institute of Marine Science and Technology of Shandong University, Qingdao, China.

† Zhao-Jie Teng, Peng Wang and Xiu-Lan Chen contributed equally to this work.

* Corresponding author: Yu-Zhong Zhang, zhangyz@sdu.edu.cn

Abstract

Cleavage of dimethylsulfoniopropionate (DMSP) can deter herbivores in DMSP-producing eukaryotic algae, however it is unclear if a parallel defense mechanism operates in marine bacteria. Here, we demonstrate that the marine bacterium *Puniceibacterium antarcticum* SM1211, which does not use DMSP as a carbon source, has a membrane-associated DMSP lyase, DddL. At high concentrations of DMSP, DddL causes an accumulation of acrylate around cells through the degradation of DMSP, which protects against predation by the marine ciliate *Uronema marinum*. The presence of acrylate can alter the grazing preference of *U. marinum* to other bacteria in the community, thereby influencing community structure.

Main text

Predation and defense are important predator-prey interactions in the microbial realm[1]. Chemical defense strategies are widespread in the marine environment[2]. Dimethylsulfoniopropionate (DMSP) is one of the most abundant organosulfur compounds in marine ecosystems[3], which is synthesized by a range of marine phytoplankton[4,5] and bacteria[6,7] with concentrations ranging from the low nanomolar in open ocean waters to hundreds of millimolar within phytoplankton[3,8]. Whilst DMSP cleavage plays an important ecological role to deter herbivores in DMSP-producing eukaryotic algae[9-12], whether a parallel system operates in marine bacteria remains elusive. Here, we report a chemical defensive strategy in marine bacteria to defend against protozoan predation with DMSP as the precursor compound.

Puniceibacterium antarcticum SM1211^{T}, isolated from Antarctic seawaters[13], was unable to grow with DMSP as the sole carbon source (Extended Data Fig. 1a, Supplementary Figure 1). When the cells of strain SM1211 were incubated in sterile seawater supplemented with 1 mM DMSP, DMS was produced at a rate of 6.06 ± 0.71 $\times 10^{-13}$ μM s^{-1} $cell^{-1}$, and acrylate was accumulated in the medium at a rate of 3.29 ± 0.34 $\times 10^{-13}$ μM s^{-1} $cell^{-1}$ (Extended Data Fig. 1b,c), indicating that the bacterium has a functional DMSP lyase. Indeed, strain SM1211 contains a single *dddL* gene without any known acrylate-utilizing genes in its genomic neighbourhood (Supplementary Figure 2). No other known DMSP lyase genes were found in its genome. Furthermore, strain SM1211 did not grow on acrylate as a sole carbon source (Extended Data Fig. 1a,d). However, it can tolerate up to 10 mM acrylate without affecting its growth in 2216E medium (Extended Data Fig. 1e).

The *dddL* gene was constitutively expressed at low level (< 3 fold induction) when strain SM1211 was induced by 1 mM concentrations of DMSP, acrylate or 3-hydroxypropionate (3-HP) (Extended Data Fig. 1f). The *dddL*-deletion mutant Δ*dddL* lost the abilities to produce DMS and to accumulate acrylate outside the cell. In contrast, the complemented strain ZH171 regained these abilities (Extended Data Fig. 1b,c,g). Analyses of the cellular location of DddL in strain SM1211 by fluorescence labelling using superfolder green fluorescent protein (sfGFP)[14] and DMSP cleavage activity assay suggest that DddL is likely membrane-associated (Extended Data Fig. 1h-j). Interestingly, the sfGFP labelled-DddL was observed to be accumulated at the cell poles (Extended Data Fig. 1k-m). Purified DddL from the membrane fraction of

recombinant *E. coli* was active against DMSP, having relatively high catalytic efficiency and affinity compared to other DMSP lyases (Extended Data Fig. 2, Supplementary Table 1).

Next, we set out to test whether DMSP catabolism to acrylate plays a defensive role to protect strain SM1211 against *Uronema marinum,* a well-studied cosmopolitan bacterivorous ciliate, which was also found in Antarctic seawaters[15] where high DMSP concentrations are observed[16]. In a short-term predation experiment, bacterial cells were pre-labelled with a fluorescent dye[17] (Extended Data Fig. 3a-d) and incubated with *U. marinum* for 5 min to avoid digestion of the bacteria within *U. marinum*[18]. Food vacuoles of *U. marinum* were then observed and enumerated (Fig. 1a). When *U. marinum* preyed upon strain SM1211 in seawater containing 500 μM DMSP, a reachable concentration within microzones surrounding damaged DMSP-producers[19], the average predation rate declined by a half compared to that without DMSP, suggesting that the addition of DMSP inhibited prey capture (Fig. 1b, Supplementary Table 2). Furthermore, the non-predation ratio increased more than four times in the presence of DMSP. This anti-grazing feature caused by DMSP disappeared in the Δ*dddL* mutant but was recovered in the complemented mutant strain ZH171. This feeding disturbance of *U. marinum* by DMSP was not observed in the negative control strain *Pseudoalteromonas arctica* which does not contain any known DMSP lyase gene (Fig. 1b).

During a long-term co-culture experiment (90 h), an approximately three-fold decrease in both maximum growth rate and net stationary phase population abundance

of *U. marinum* was observed when strain SM1211 or the complemented mutant strain ZH171 was fed in the presence of 500 μM DMSP, compared to that without DMSP (Fig. 1c, Supplementary Table 3). This inhibitory effect was observed in each growth phase of the *U. marinum* population. Moreover, this defense mechanism could be observed in DMSP as low as 5 μM (Supplementary Table 4), a concentration that can occur at algal cell surface due to lysis or grazing[19]. In contrast, the predation rate and the population abundance of *U. marinum* feeding on either Δ*dddL* or *P. arctica* rose slightly in the presence of 500 μM DMSP (Fig. 1c), indicating that DMSP is not the direct defense compound but the chemoattractant (Extended Data Fig. 3e). Acrylate was detected at micromolar concentrations in the DMSP-containing co-culture of *U. marinum* with strain SM1211 or ZH171, but not in that with Δ*dddL* nor *P. arctica*, suggesting that DMSP catabolites could play a defense role. *U. marinum* showed a significant attraction towards DMS at micromolar concentrations, and its cells increased when 500 μM DMS was added to the *U. marinum*:Δ*dddL* co-culture (Extended Data Fig. 3f-g), demonstrating the role of DMS as an appetizing signal[20]. However, the addition of 70 μM acrylate to this co-culture significantly reduced the *U. marinum* population growth (Extended Data Fig. 3h-i). In addition, the acrylate concentration declined with the population growth of *U. marinum* preying on inactivated *P. arctica*, indicating a likely acrylate consuming process by *U. marinum*. Given that the acrylate concentration in the co-culture was much lower than the lethal concentration of acrylate to *U. marinum* (1 mM, Extended Data Fig. 3j), the possibility that the reduction in *U. marinum* cell numbers in this experiment due to

112 acrylate toxicity can be excluded. Together, these results indicate that when DMSP (≥
113 5 μM) is present, acrylate produced from DMSP cleavage by strain SM1211 via the
114 membrane-associated DddL acts as a predation deterrent to *U. marinum*.

115 To determine the role of DMSP on selective grazing by *U. marinum*, a dual prey
116 system was set up using strains SM1211 and its mutant Δ*dddL* (Fig. 2a, Extended
117 Data Fig. 4a). In the presence of 500 μM DMSP, *U. marinum* selectively grazed on
118 Δ*dddL* over SM1211 whereas in the absence of DMSP, no selectivity was observed
119 (Fig. 2b, Supplementary Table 5), indicating that predation pressure from *U. marinum*
120 on strain SM1211 is largely transferred to Δ*dddL* in the presence of 500 μM DMSP.
121 Since acrylate (5 mM) had no obvious impact on the fitness of Δ*dddL* (Extended Data
122 Fig. 4b), the decreased predation of *U. marinum* on strain SM1211 in the presence of
123 500 μM DMSP indicates that the *dddL* gene confers strain SM1211 an advantage in
124 intra-species competition due to acrylate being a grazing deterrent.

125 We also assessed selective predation using the wild-type or Δ*dddL* mutant of
126 SM1211 and *P. arctica* which can tolerate up to 10 mM acrylate (Extended Data Fig.
127 4c-d), and had no intracellular acrylate accumulation (Supplementary Table 6). In the
128 presence of 500 μM DMSP, strain SM1211, but not the Δ*dddL* mutant, became less
129 palatable for *U. marinum,* leading to a pronounced increase in the population ratio of
130 SM1211:*P. arctica* after 72 h predation (Fig. 2c, Supplementary Table 7). Such an
131 increase was not observed in the absence of DMSP or *U. marinum*. Moreover, we
132 simulated a four species prey community and monitored their interaction with *U.*
133 *marinum*. In addition to strain SM1211, the Δ*dddL* mutant and *P. arctica*, another two

non-*dddL* containing strains (*Marinobacter antarcticus* 4-2 and *Polaribacter* sp. K15) were used. These two strains neither accumulate acrylate nor grow on DMSP as a sole carbon source, although they can tolerate acrylate up to 1 mM (Extended Data Fig. 4e-h, Supplementary Table 6). Similarly, the relative abundance of SM1211, but not the Δ*dddL* mutant, increased significantly in the presence of 500 μM DMSP (Fig. 2d, Extended Data Fig. 4i, Supplementary Table 8,9). These results suggest that the grazing protection provided by acrylate from DMSP cleavage by DddL shifts the predation preference and grazing pressure of *U. marinum* from strain SM1211 to non-*dddL*-containing bacteria. With this defense strategy, strain SM1211 not only gains a competitive advantage over other palatable strains in terms of nutrients and space, but also reduces its mortality.

Searches on samples from the IMG/M database[21] showed that DddL homologues are widely distributed both geographically and phylogenetically (Extended Data Fig. 5), suggesting that DddL-dependent grazer deterrent is not unique to SM1211. Therefore, we also investigated interaction of *U. marinum* with other 4 *dddL*-containing strains and *Ruegeria lacuscaerulensis* ITI-1157 that contains DMSP lyase genes *dddP*[22] and *dddQ*[23] but not *dddL* in its genome (Supplementary Table 6, Extended Data Fig. 6a-b). The results indicate that this DMSP-dependent defense strategy appears to offer protection for other *dddL*-containing bacteria that do not use DMSP as carbon source (Extended Data Fig. 6c-g).

In summary, we show an inherent defense strategy performed by a DddL-containing bacterium through conversion of high concentrations of DMSP into

acrylate (Fig. 1,2). This protection strategy likely enables *dddL*-containing bacteria that do not use DMSP as carbon source to gain a survival advantage, but moreover influences bacterial community structure by shifting the predation pressure to non-*dddL* containing bacteria (Extended Data Fig. 7).

Methods

Strains and growth conditions

Strains used and constructed in this study are shown in Supplementary Table 10. *Puniceibacterium antarcticum* SM1211[13] and *Pseudoalteromonas arctica* (*P. arctica*) were isolated from Antarctic surface seawater (62°12′16″ S, 58°56′19″ W; 62°14′05″ S, 58°54′11″ W). *Marinobacter antarcticus* 4-2 and *Polaribacter* sp. K15 were isolated from Antarctic intertidal sediment (62°11′32″ S, 58°58′34″ W) and a rotten seaweed (62°11′50″ S, 58°59′37″ W), respectively. All bacterial strains were cultured at 25°C in 2216E medium[24]. *Escherichia coli* strains (Supplementary Table 10) were grown in Luria-Bertani (LB) medium at 37°C. Bacterial growth was monitored automatically using an automatic growth curve analyzer (Bioscreen C MBR, Finland).

Uronema marinum was isolated from coastal seawater off Qingdao, China, and cultured at 18□ in artificial seawater filtered through a 0.22 μm pore size Millex-GP filter (Millipore, USA), supplemented with 1‰ vitamins and 1% ultrasonic broken *E. coli* BL21 (DE3) cells (OD_{600} = 2.0) as prey[15]. Artificial seawater was prepared using sea salts (pH 7.8-8.0, salinity 35‰, Sigma-Aldrich, USA).

To examine the acrylate tolerance of the strains, each strain was cultured at 25°C for 120 h in 2216E medium supplemented with 0, 1 mM, 5 mM or 10 mM acrylate (Sigma, USA). To evaluate the microbial competition between strain SM1211 and Δ*dddL*, a mix of strains ZH176 and ZH177 (OD_{600} = 0.25 of each) was plated on 2216E solid medium containing 50 μg mL^{-1} gentamycin to enumerate Δ*dddL* over 3 days. For sole carbon source experiments, strain SM1211 were cultured to an OD_{600} of 1.0 and washed 3 times in sterile artificial seawater. Subsequently, a 1% (v/v) strain inoculum was transferred to minimal medium[25] containing 1‰ vitamin solution[25] and grown at 25°C for 60 h with or without succinate, DMSP (TCI, Japan) or acrylate at a concentration of 1 mM as the sole carbon source.

Gene amplification and construction of plasmids and mutants

Vector pK18mobsacB-Ery[26] and pBBR1MCS-4[27] was used for gene knockout and gene complementation, respectively (Supplementary Table 10). The *dddL* gene, a 412 bp fragment of the promoter region of the *dddL* gene (P_{ddl}), and two 500 bp fragments upstream and downstream of *dddL* (for constructing homo-*dddL* overlaps) were cloned from genomic DNA of strain SM1211 with the primers listed in Supplementary Table 11. Superfolder green fluorescent protein (sfGFP)[14,28] was used for protein localization.

The gene knockout mutant Δ*dddL*, the recombinant strains ZH171 to ZH177 were constructed and selected by growth on 2216E solid medium containing relevant antibiotics (Supplementary Table 10)[26,29]. Antibiotics were used at the following concentrations: ampicillin, 70 μg mL^{-1} for strains ZH171 to ZH175 and 100 μg mL^{-1}

for *E. coli*; kanamycin, 50 μg mL^{-1} for strain ZH176; gentamycin, 50 μg mL^{-1} for strain ZH177. All mutants were verified via DNA sequencing (TsingKe, China).

Examination of the localization of DddL

To examine the localization of DddL, strains ZH173, ZH174 and ZH175 were cultured to stationary phase in 100 mL 2216E medium containing 1 mM DMSP, and then observed under a Leica LSM TCS-SP8 confocal laser scanning microscope (Leica, Germany) with excitation wavelengths of 450-490 nm. The periplasmic, membrane and cytoplasmic fractions of strain ZH173 were prepared via ice cold 5 mM $MgSO_4$[14]. The fluorescence density of each fraction was measured using a Fluorolog spectrofluorometer (Horiba Jobin Yvon, Japan). The fluorescence density of 5 mM $MgSO_4$ was used as a control. To measure DMSP cleavage activity, the periplasmic, membrane and cytoplasmic fractions were mixed with 1 mM DMSP, respectively, and incubated in reaction buffer containing 100 mM Tris-HCl (pH 8.0) in a total volume of 1 mL at 18°C for 11 h. Protein concentration was determined using the BCA assay[30].

Assay of extracellular acrylate production

Strains were cultured to stationary phase at 25□ in 2216E medium containing 1 mM DMSP. Strains cultured without DMSP were used as the control. Each culture sample was washed 3 times in sterile seawater and resuspended in 1 mL sterile artificial seawater containing 1 mM DMSP. Then, 100 μL of each sample was fixed with 100 μL 10% formaldehyde and stored at 4°C for enumeration via flow cytometry. The remainder of the culture was incubated at 18°C for 24 h to metabolize DMSP. After

incubation, cells were inactivated using 200 μL perchloric acid and acrylate concentrations in the supernatant were detected by high performance liquid chromatography (HPLC, Prominence LC-20AD, SHIMADZU, Japan) on a Sunfire C18 column (Waters, Ireland) which was operated with HPLC buffer[31] (2.5% acetonitrile, 0.2% phosphoric acid in double-distilled H_2O) at a flow rate of 1 mL min^{-1}.

DMS production assays

DMS production was determined by GC analysis on a gas chromatograph (GC-2030, Shimadzu, Japan) equipped with a flame photometric detector[32]. Strains were first cultured in 2216E medium at 25°C to an OD_{600} of 1.0, and then washed 3 times with sterile seawater. The strain samples were diluted 1:10 in sterile seawater supplied with 1 mM DMSP, and sealed in 5 mL vials (Anpel, China). The mixture was incubated at 18°C for 24 h before GC analysis on a gas chromatograph (GC-2030, Shimadzu, Japan) equipped with a flame photometric detector[32]. An eight-point calibration curve of DMS standards was used[6]. To measure the DMSP content in artificial seawater, 1 mL seawater was placed in a 5 mL vial with 100 μL KOH (10 M) at 18°C for 1 h before GC analysis of DMS.

Real-time qPCR analysis of *dddL* transcription

Strain SM1211 was cultured to an OD_{600} of 1.0 and washed 3 times in sterile artificial seawater, and then inoculated at 1% (v/v) in minimal medium containing 10 mM succinate as the sole carbon source. Cells were grown at 25°C for 48 h until the OD_{600} reached 0.1. DMSP, acrylate or 3-HP (final concentration of 1 mM) was added to

induce *dddL* expression. Controls were also set up with 10 mM succinate as the carbon source. Cells (1 mL) were collected for analysis after 0.5 h, 1 h, 2 h, 4 h and 6 h induction. RNA was extracted using the RNeasy mini kit (QIAGEN, Germany) and qPCR were performed using a Light Cycler II 480 System (Roche, Switzerland)[33]. The *rpoD* gene was used to normalise the expression of *dddL*. Expression levels of *dddL* in strain SM1211 with different inducers at each sampling time were normalized by that without inducer. The qPCR primers were listed in Supplementary Table 11.

Heterologous expression and purification of the DddL protein

The *dddL* gene from strain SM1211 was cloned into pET22b (Novagen, USA) with a C-terminal His tag, and the recombinant plasmid was transferred into *E. coli* strain C43 (DE3)[34]. Recombinant *E. coli* cells were cultured at 37°C in LB medium to an OD_{600} of 0.8-1.0 and then induced at 20°C for 16 h with 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Cells were then lysed by a high pressure cracker and cell membranes were isolated from the disrupted cells by ultracentrifugation, solubilized with 40 mM dodecylmaltoside (DDM), and fractionated by gel filtration on a Superdex G75 column (GE Healthcare, USA)[35]. The purified DddL, and the cytoplasmic and membrane fractions were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting.

DMSP lyase assays

To measure DddL enzymatic activity, DddL (final concentration of 0.3 μM) and DMSP (final concentration of 1 mM) were mixed in the reaction buffer containing 100 mM Tris-HCl (pH 8.0) in a total volume of 100 μL. After the mixture was

incubated at 30°C for 20 min, the reaction was stopped using perchloric acid. To determine the optimal temperature for DddL activity, reaction mixtures were incubated at 4°C, 18°C, 30°C, 40°C, and 60°C. The optimum pH for DddL activity was examined using Britton-Robinson buffer[36] at pH values of 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0. After the optimal pH and temperature were determined, all measurements of DddL activity were performed under the optimal pH and temperature. Kinetic parameters of DddL were determined based on the initial rates determined with 0.3 μM DddL and 0.5-9 mM DMSP at 4°C, pH 8.0 (Antarctic conditions); 18°C, pH 8.0 (general ocean conditions); or 30°C, pH 9.0 (optimal conditions).

Short-term predation experiments

Short-term predation contains encounter and capture processes. Strains SM1211, Δ*dddL*, ZH171 and *P. arctica* were cultured at 25°C to stationary phase in 2216E medium supplemented with 1 mM DMSP. A 1 mL culture sample of each strain was then washed 3 times with sterile seawater, resuspended in 200 μL 1 mM 5-(4,6-dichlorotriazinyl) aminofluorescein[17] (DTAF, Thermofisher, USA), and incubated at 4°C overnight for fluorescently labelled bacteria (FLB) preparation. After labelling, FLB samples were washed 3 times, resuspended in 1 mL 0.22 μm pore size filtered artificial seawater, and incubated at 18°C with or without 500 μM DMSP for 6 h before being used for predation experiments[37]. *U. marinum* cells were washed 3 times with sterile seawater to remove its food source and starved at 18°C in the dark for 24 h before being used for predation experiments. Approximately 50 starved ciliates were added to each FLB sample with a ratio of ciliate to FLB being ~1:10^6 [38] in the

presence or absence of DMSP. Ciliates were collected manually after 5 min ingestion and fixed with 4% (v/v) glutaraldehyde instantly before ciliate food vacuoles became acidified for FLB digestion[18,39]. Food vacuoles from *n* = 135-268 ciliates were counted (Supplementary Table 2) under a fluorescence microscope (Olympus BX61, Japan). Statistical analysis was carried out using SPSS 17.0 (IBM, USA). A two-sided *t*-test was used to analyse whether ciliate predation rates and ratios were statistically significantly different.

Long-term growth experiments

Long-term predation contains encounter, capture, ingestion and digestion processes. Predation systems were set up using 30 mL co-cultures incubated in 25 cm^2 cell culture flasks (Corning, USA) at 50 rpm and 18°C[38]. Approximately 300 ciliate cells were added to the predation system and the ratio of ciliates to bacteria was ~1:10^6.

All strains were grown to stationary phase at 25□ in 2216E medium containing 1 mM DMSP, which were then suspended in 0.22 μm pore size filtered artificial seawater and adjusted to an OD_{600} = 0.60. *U. marinum* was pre-cultured to early stationary phase and then incubated with bacterial strains with or without 500 μM DMSP at 18□ for 90 h. Strains SM1211 and Δ*dddL* were used to perform long-term growth experiments with or without 70 μM acrylate at 18□ for 90 h. Strain Δ*dddL* were also used to perform long-term growth experiments with or without 500 μM DMS (Sigma-Aldrich, USA) at 18□ for 90 h. To determine the minimum effective concentration of DMSP for strain SM1211 to defend against ciliate predation, 0, 5 μM, or 10 μM DMSP was added to the predation system containing strain SM1211

and *U. marinum*, which was incubated at 18°C for 70 h. To determine the minimum lethal concentration of acrylate to *U. marinum*, 0, 70 μM, or 1 mM acrylate was added to the predation system containing inactivated *P. arctica* and *U. marinum*, which was incubated at 18°C for 80 h. Subsamples (450 μL) were taken from batch cultures every 6 h and fixed with formaldehyde solution (1 % v/v) at 4°C for 24 h. Ciliates in the fixed subsamples were counted for 5 min, and 10 to 500 objects were recorded via flow cytometry[40] according to the different growth phases of the *U. marinum* population. Growth curves of the ciliate were fitted to a hyperbolic function equivalent to a Growth/Sigmoidal function $y = N/(1 + ((a-N_0)/N_0)*e^{(-4*\mu_{max}*x/N)})$ using OriginPro 8.

Chemoattraction of *U. marinum* to DMSP and DMS

To test the chemotactic responses of *U. marinum* to DMSP and DMS, a microfluidic biochip was constructed, in which one ciliate pool and one chemotactic compound pool was directly connected via several channels. The size of each microchannel was 60 mm × 250 μm × 30 μm (length × width × height). Approximately 100 *U. marinum* cells were added to the ciliate pool for DMSP and DMS chemoattraction assays. The times of *U. marinum* in the ciliate pool attracted into the microchannels with or without 500 μM DMSP or 100 μM DMS in the chemotactic compound pool were recorded for 10 min under an inverted microscope (Nikon Ts2, Japan). After 10 min, the number of *U. marinum* cells detained in microchannels and the chemotactic compound pool were counted.

Selective predation experiments

Intra-species selective predation experiments were performed as described for the short-term predation experiments with slight modification. In brief, strains ZH176 and ZH177 were cultured to stationary phase, washed 3 times in sterile seawater and incubated at 18□ for 6 h with or without 500 μM DMSP before being used for predation experiments. *U. marinum* cells were washed 3 times in sterile seawater to remove its food source and starved at 18□ in the dark for 24 h. Starved ciliates (n = 10,000) were added to bacterial samples in a 24 well microplate, and the ratio of ciliates:bacteria in each sample well was approximately $2:10^4$ or a multiplicity of infection (MOI) of 5,000[38]. For strains ZH176 and ZH177, each bacterial strain was inoculated at an MOI of 2,500 in order to have a global MOI of 5,000 between bacteria and protozoa. After 5 min of interaction, the suspensions were fixed using 3.5% (v/v) formalin for 30 min, and then a coverslip previously coated with poly-L-Lysin was deposited in each well. Low speed centrifugation (15 min at 900 g) was used to initiate and increase the adhesion of the cells to the coverslip. Cells were then stained with 5 mg/mL DAPI and the coverslips were mounted with a drop of Mowiol antifade before observation. At least 100 ciliates per biological replicate (with at least one food vacuole, n = 475) and their interior food vacuoles (n = 618) were then observed and counted (Supplementary Table 5) using a Laser scanning confocal microscope LSM 880 (CLSM, Zeiss, Jena, Germany). Statistical analysis was carried out using SPSS 17.0. A two-sided t-test was used to analyse whether ciliate predation rates and ratios were statistically significantly different.

For interspecies selective predation experiments, the mixture of *P. arctica* (or *P. arctica, Marinobacter antarcticus* 4-2 and *Polaribacter* sp. K15) with strain SM1211 or Δ*dddL* (OD_{600} = 0.6 of each) was used as prey and added to cultures with or without 500 μM DMSP and in the presence or absence of ciliates and incubated at 18□ for 72 h. The mixture of *P. arctica* with strain SM1211 or Δ*dddL* was also pre-incubated for 10 h before being used as prey. Approximately 300 ciliate cells were added to the predation system and the ratio of ciliates:bacteria was ~1:10^6. The original mixtures of two or four bacterial strains at 0 h and those after 72 h culture with or without ciliates were filtered onto 0.22 μm pore size membrane filters (Merk, USA), respectively. The bacterial DNA on the membranes was extracted via DNeasy PowerWater Kit (QIAGEN, Germany). RT-qPCR was performed for community composition analysis. The primers of the above mentioned strains were listed in Supplementary Table 11.

Enumeration of bacteria and ciliates

Flow cytometry was used to count bacterial cells used for DMS and acrylate analyses as well as ciliates from long-term growth experiments. Cells were stained with SYBR Green I (Solarbio, China) at a ratio of 10,000:1 at room temperature for 30 min in the dark[40] and enumerated on an Imaging Flow Cytometer (Amnis ImageStream®X Mark II, USA) using INSPIRE software. The instrument and INSPIRE software were set up as follows: channel 01, bright field; channel 02, fluorescence channel; 40 times magnification; excitation wavelength, 488 nm; and flow rate, low speed/high sensitivity (0.16 μL s^{-1}). To obtain a collection gate, a scatter plot of channel 02

fluorescence intensity was plotted against the channel 02 area (Supplementary Figure 3). Bacteria and ciliate gates were circled according to the images. For bacteria, cells were diluted to an OD_{600} of 0.1 and 20,000 cells were counted. For the ciliate, 48 μL (0.16 μL s^{-1} × 300 s) of each ciliate sample was used for ciliate enumeration. IDEAS software was used to determine the concentration of the bacteria or ciliates[41].

Modelled accumulating and diffusing acrylate patches

A 1 μm radius SM1211 cell, with an internal acrylate generating rate of 3.29×10^{-13} μmol s^{-1} $cell^{-1}$ (Supplementary Table 6), was modelled by solving the spherically symmetric diffusion equation for the acrylate concentration C, $\partial C/\partial t = (D/r^2)\ \partial/\partial r(r^2 \partial C/\partial r) + S$, where t is time, r is the radial distance from the center of the bacteria cell, D is the diffusivity of acrylate, and S is the source term representing the average generating rate of acrylate per unit volume in a strain SM1211 cell (r_0 = 1 μm). The diffusion coefficient for acrylate at 18º C was $D = 10.14 \times 10^{-10}$ m^2 s^{-1} that was computed[42] using the molar volumes with 79.13 cm^3 mol^{-1} for acrylate[43]. $S = 7.85 \times 10^{-10}$ μM s^{-1} m^{-3}, when $r \leq r_0$; S = 0, when $r > r_0$. The initial condition was 0 for the acrylate concentration in a SM1211 cell, with the half-width of 1 μm and acrylate generating rate equal to that of strain SM1211.

Bioinformatics analysis

The amino acid sequences of DddL (*Puniceibacterium antarcticum*, WP_099909581.1) were used to search against all genomes and selected metagenomes (Supplementary Table 12,13) on the IMG/M metagenomics database[21] with the parameters of e-value $<10^{-30}$ and identity > 40%[20]. Biofilm samples were

from Zhang *et al.*[44] The neighbor-joining trees of the *dddL*-harboring strains were constructed using MEGA 6.0 with 1000 bootstraps and modified via iTOL (https://itol.embl.de/). Invariant amino acid residues were identified using BioEdit 7.0.5.3. Conserved motifs were predicted by MEME 5.0.5[45] and the *trans*-membrane region predicted by TM prep[46]. Horizontal gene transfer events were predicted via RANGER-DTL 2.0[47].

Data Availability: Sequence data that support the findings of this study are available in the Supplementary information.

Code Availability: No custom code was used.

Statistics & Reproducibility: No statistical method was used to predetermine sample size but our sample sizes are similar to those reported in previous publication[12]. Data distribution was assumed to be normal but this was not formally tested. No data were excluded from the analyses. The experiments were not randomized. Data collection and analysis were not performed blind to the conditions of the experiments.

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Author Contributions Statement: Z.-J.T. designed and performed the majority of the experiments and data interpretation. P.W. conceived the project and performed protein purification. X.-L.C. directed the study. R.G. performed the intraspecies selective predation experiments and data interpretation. C.-Y.L. helped in experiments and data interpretation. S.-B.Z. and J.G. helped in experiments. K.-W.X. modelled the micropatch diffusion dynamic of acrylate. Z.-J.T. and X.-L.C. wrote the manuscript. D.J.S. provided input in the selective predation experiments and critically revised the manuscript. Y.C. directed the study and did a critical revision of the manuscript for important intellectual content. Y.-Z.Z. conceived the project, designed the experiments, and directed the study.

Competing Interests Statement: Authors declare no competing interests.

Figure Legends

Fig. 1 | The predation rates and ratios of *Uronema marinum* preying on the wild-type *Puniceibacterium antarcticum* SM1211 and the *dddL*-deletion mutant Δ*dddL* in the presence and absence of DMSP.

a, Representative food vacuoles (white vacuoles) formed in *U. marinum* feeding on strain SM1211, the mutant Δ*dddL*, the complemented mutant ZH171 and *Pseudoalteromonas arctica* (*P. arctica*) in the presence or absence of 500 μM DMSP. Scale bars = 10 μm. Each picture is a representative of at least three repeats. **b,** Predation rates and ratios of *U. marinum* preying on strains SM1211 (n = 197 and 151, P value = 1.18E-11), Δ*dddL* (n = 135 and 186, P value = 0.081), ZH171 (n = 268 and 193, P value = 5.61E-23) and *P. arctica* (n = 152 and 136, P value = 0.109) in the presence or absence of 500 μM DMSP. The boxes in the plot are bound by the 25% to 75% quartile proportions with the thick line being the median value. Red dots indicate average values. A two-sided t-test was used to assess statistically significant differences of ciliates preying on strain in the presence or absence of DMSP; **, P value < 0.01; ***, P value < 0.001; n, the number of ciliates. **c**, Growth curves of *U. marinum* feeding on strains SM1211, Δ*dddL*, ZH171 and *P. arctica* in the presence or absence of 500 μM DMSP. The dotted red lines indicate the concentration of acrylate in the medium with DMSP. No acrylate was detected in the medium without DMSP. The acrylate concentration on the y-axis is shown in red. Approximately 300 ciliate cells were added to the predation system and the ratio of ciliates to bacteria was ~1:10^6. All experiments were carried out in three replicates.

Fig. 2 | Decrease of protozoan predation preference to *Puniceibacterium antarcticum* SM1211 in the presence of DMSP.

a, Representative confocal micrographs of the food vacuoles in *Uronema marinum* preying on the co-incubated strain SM1211 and the mutant Δ*dddL* in the absence or presence of 500 μM DMSP. Strain SM1211 and the mutant Δ*dddL* were labelled with sfGFP and mCherry, respectively, and the nucleus of *U. marinum* was stained by DAPI. Scale bars = 10 μm. Each picture is a representative of at least three repeats. **b,** The percentage of *U. marinum* preying on the co-incubated strain SM1211 (n = 61 and 48, P value = 0.002), the mutant Δ*dddL* (n = 77 and 100, P value = 0.054) or both strains (n = 61 and 128, P value = 0.084) in the absence or presence of 500 μM DMSP. A two-sided t-test was used to assess statistically significant differences between samples with different treatment; **, P value < 0.01. n, the number of ciliates. Data are presented as mean values +/- standard deviation. Error bars represent standard deviation of triplicate experiments. **c-d,** Prey abundance ratios in the presence or absence of 500 μM DMSP with or without *U. marinum* after 72 h incubation. Two bacterial species (**c,** strain SM1211 or the mutant Δ*dddL* with *Pseudoalteromonas arctica*) or four bacterial species (**d,** strain SM1211 or the mutant Δ*dddL* with *P. arctica*, *Marinobacter antarcticus* 4-2 and *Polaribacter* sp. K15) were utilized as prey for *U. marinum*. The dotted lines indicate the original prey abundance ratio at 0 h. Approximately 300 ciliate cells were added to the predation system and the ratio of ciliates to bacteria was ~1:10^6. All experiments were carried out in three

replicates. Data are presented as mean values +/- standard deviation. Error bars represent standard deviation of triplicate experiments.

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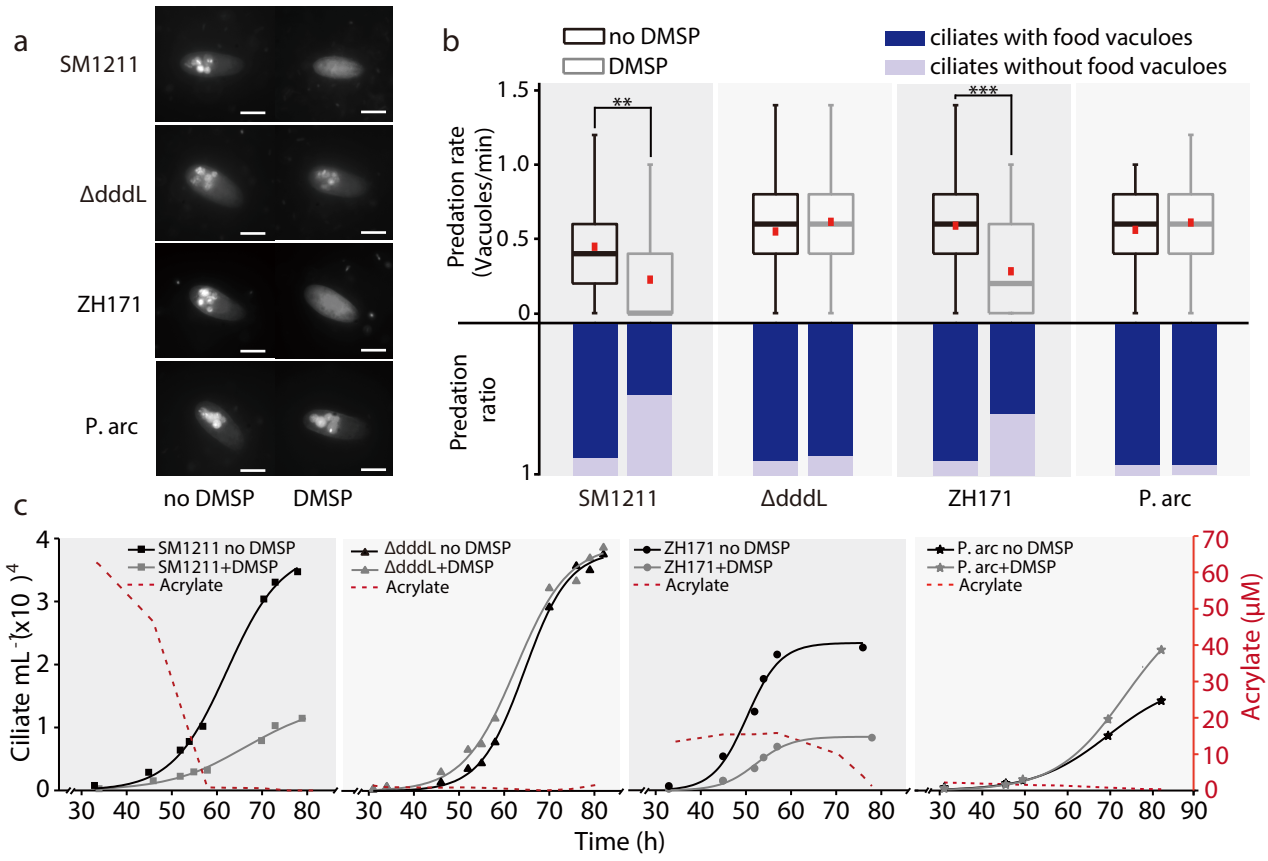
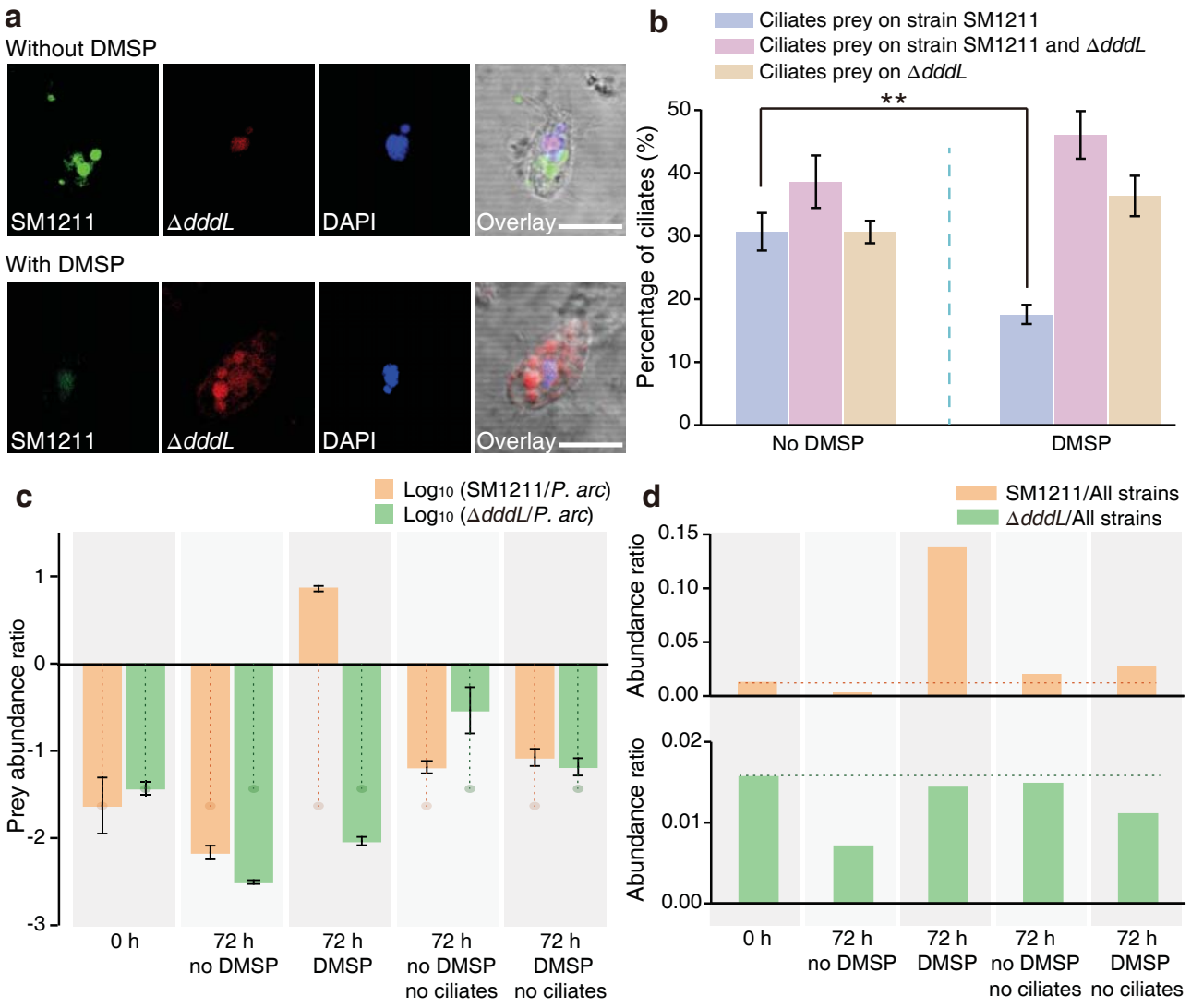
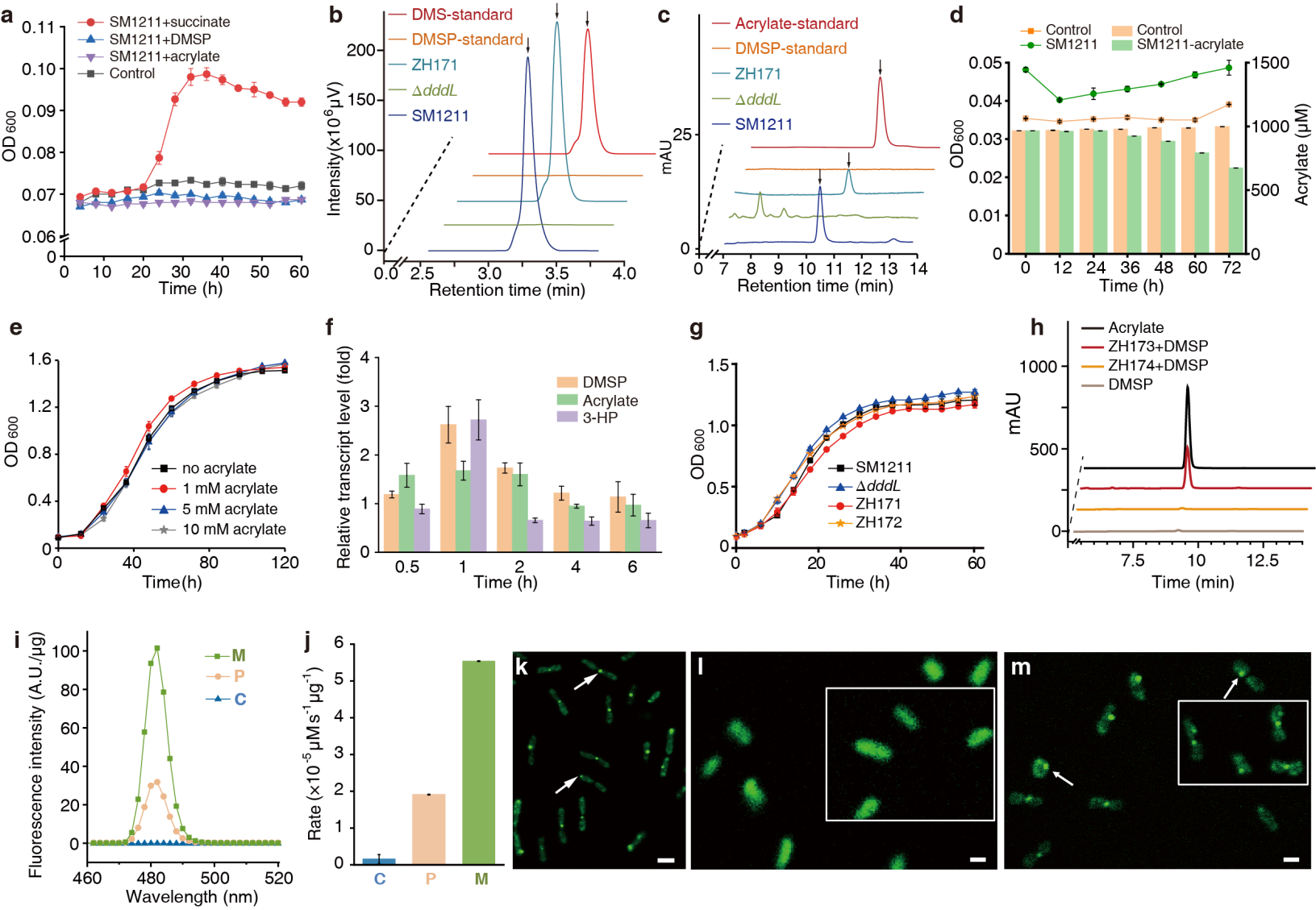
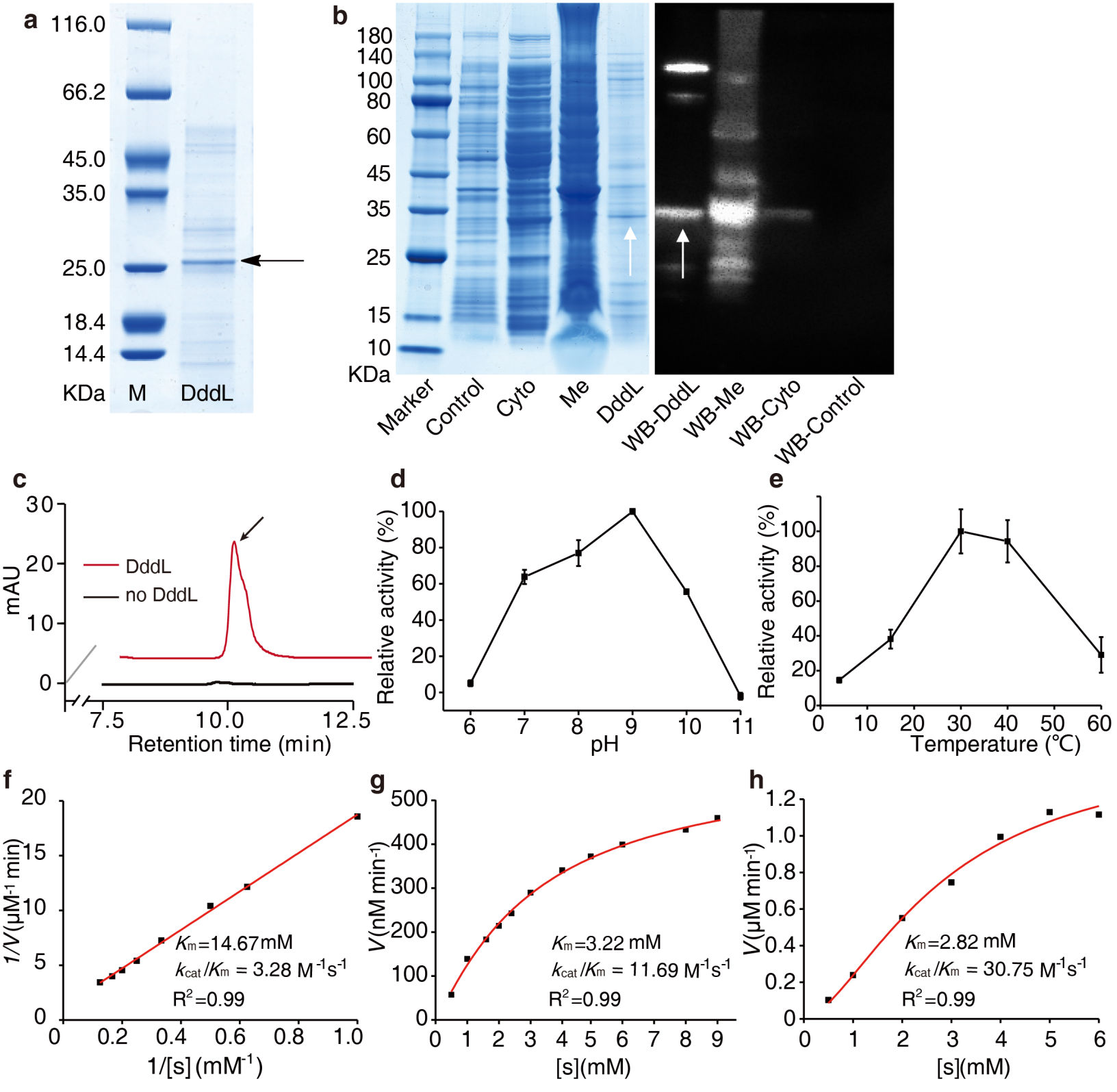
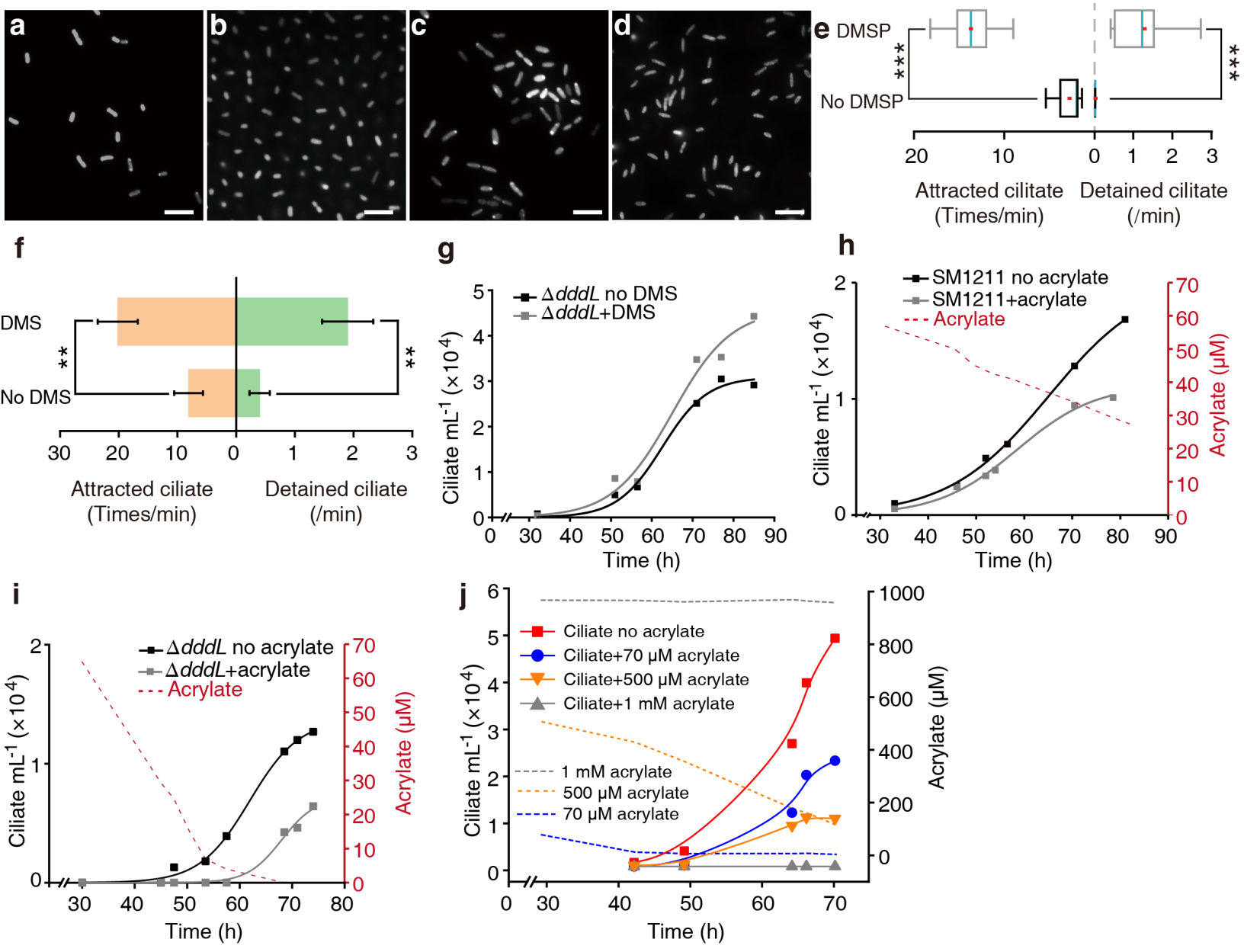
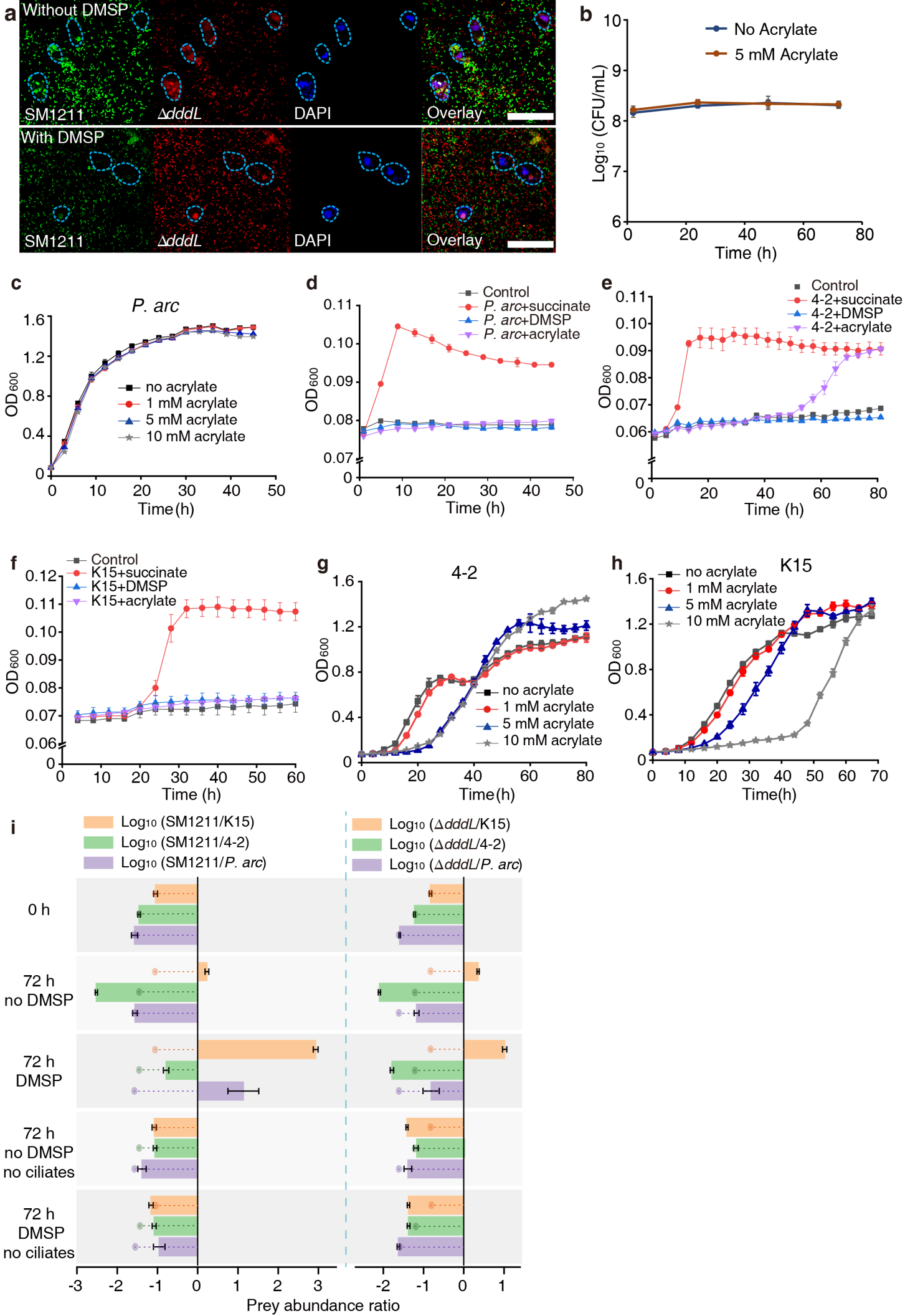

a
SM1211
ΔdddL
ZH171
P. arc
no DMSP
DMSP
b
no DMSP
DMSP
ciliates with food vaculoes
ciliates without food vaculoes
Predation rate (Vacuoles/min)
Predation ratio
**
***
SM1211
ΔdddL
ZH171
P. arc
c
Ciliate mL-1(x10)4
Acrylate (μM)
Time (h)
SM1211 no DMSP
SM1211+DMSP
Acrylate
ΔdddL no DMSP
ΔdddL+DMSP
Acrylate
ZH171 no DMSP
ZH171+DMSP
Acrylate
P. arc no DMSP
P. arc+DMSP
Acrylate

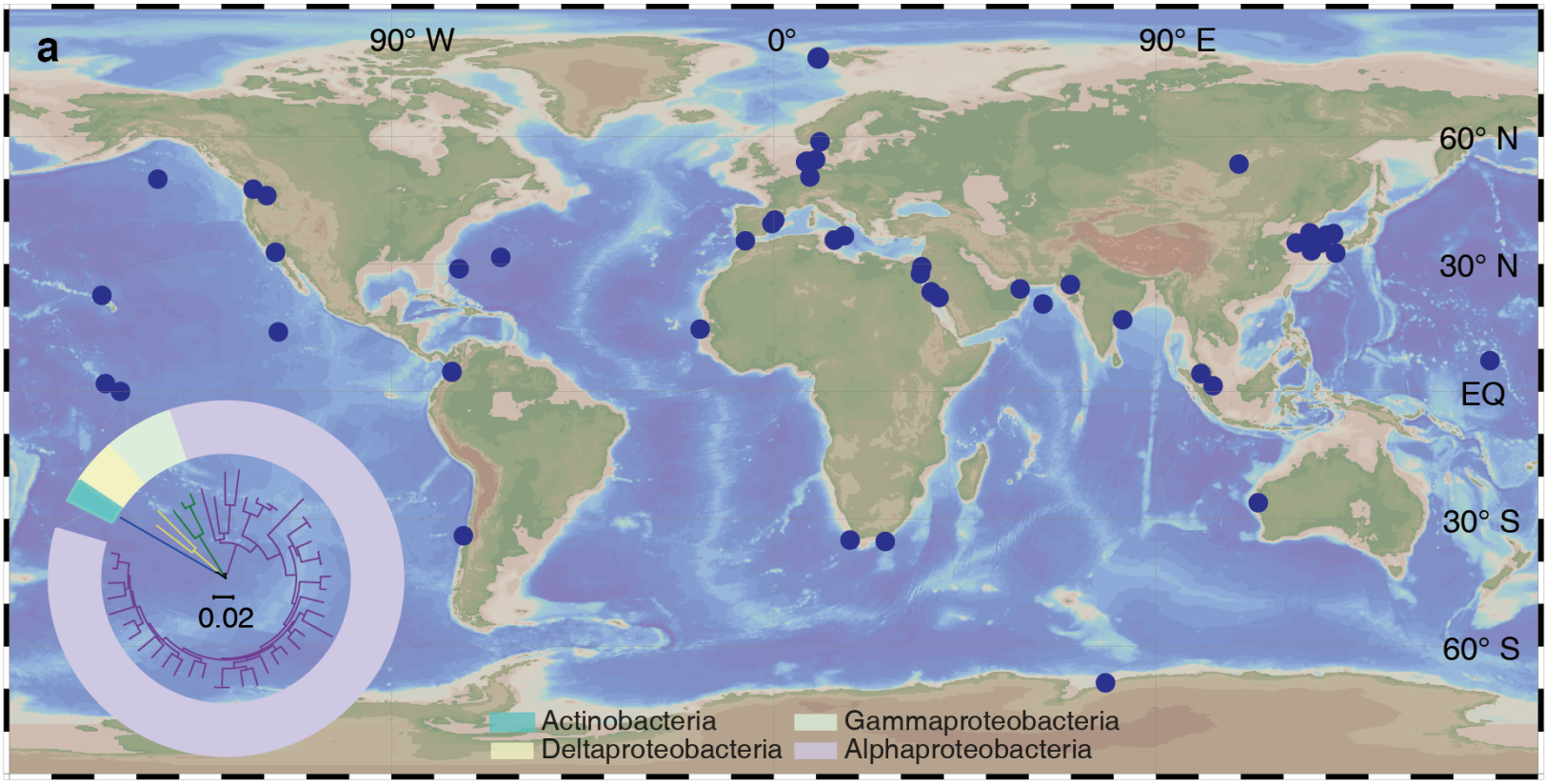

a
Without DMSP
SM1211
ΔdddL
DAPI
Overlay
With DMSP
SM1211
ΔdddL
DAPI
Overlay
b
Ciliates prey on strain SM1211
Ciliates prey on strain SM1211 and ΔdddL
Ciliates prey on ΔdddL
**
Percentage of ciliates (%)
50
40
30
20
10
0
No DMSP
DMSP
c
Log10 (SM1211/P. arc)
Log10 (ΔdddL/P. arc)
Prey abundance ratio
1
0
-1
-2
-3
0 h
72 h no DMSP
72 h DMSP
72 h no DMSP no ciliates
72 h DMSP no ciliates
d
SM1211/All strains
ΔdddL/All strains
Abundance ratio
0.15
0.10
0.05
0.00
Abundance ratio
0.02
0.01
0.00
0 h
72 h no DMSP
72 h DMSP
72 h no DMSP no ciliates
72 h DMSP no ciliates

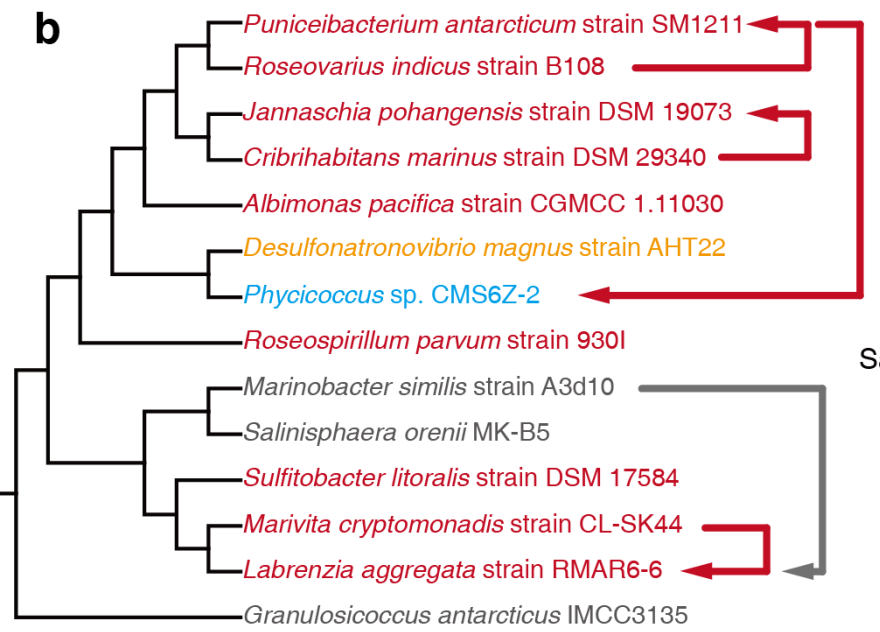

a
SM1211+succinate
SM1211+DMSP
SM1211+acrylate
Control
OD600
Time (h)
b
DMS-standard
DMSP-standard
ZH171
ΔdddL
SM1211
Intensity(x10^6 μV)
Retention time (min)
c
Acrylate-standard
DMSP-standard
ZH171
ΔdddL
SM1211
mAU
Retention time (min)
d
Control
SM1211
Control
SM1211-acrylate
OD600
Acrylate (μM)
Time (h)
e
no acrylate
1 mM acrylate
5 mM acrylate
10 mM acrylate
OD600
Time(h)
f
DMSP
Acrylate
3-HP
Relative transcript level (fold)
Time (h)
g
SM1211
ΔdddL
ZH171
ZH172
OD600
Time (h)
h
Acrylate
ZH173+DMSP
ZH174+DMSP
DMSP
mAU
Time (min)
i
M
P
C
Fluorescence intensity (A.U./μg)
Wavelength (nm)
j
Rate (×10^-5 μMs^-1μg^-1)
C
P
M
k
l
m

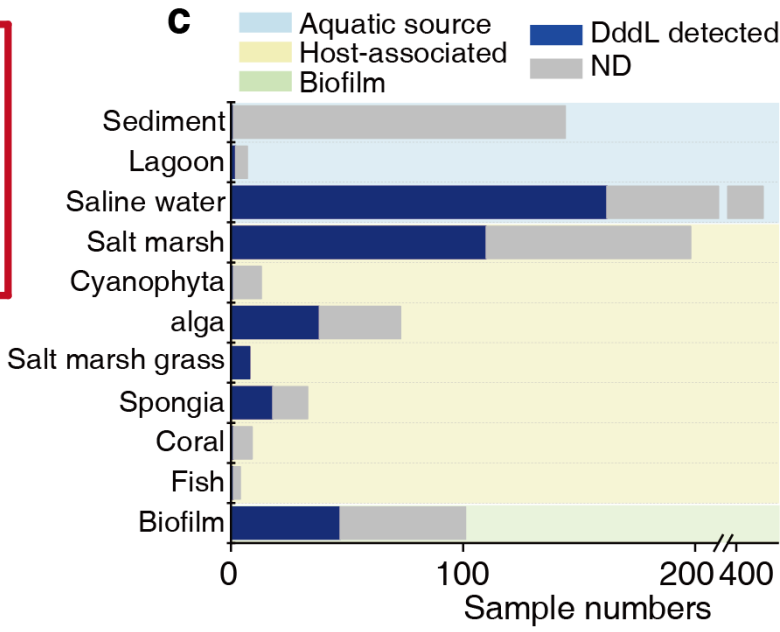

a
116.0
66.2
45.0
35.0
25.0
18.4
14.4
KDa
M
DddL
b
180
140
100
80
60
45
35
25
15
10
KDa
Marker
Control
Cyto
Me
DddL
WB-DddL
WB-Me
WB-Cyto
WB-Control
c
mAU
DddL
no DddL
Retention time (min)
d
Relative activity (%)
pH
e
Relative activity (%)
Temperature (℃)
f
1/V(μM-1 min)
1/[s] (mM-1)
Km=14.67mM
kcat /Km = 3.28 M-1s-1
R2=0.99
g
V(nM min-1)
[s](mM)
Km=3.22 mM
kcat /Km = 11.69 M-1s-1
R2=0.99
h
V(μM min-1)
[s](mM)
Km=2.82 mM
kcat /Km = 30.75 M-1s-1
R2=0.99

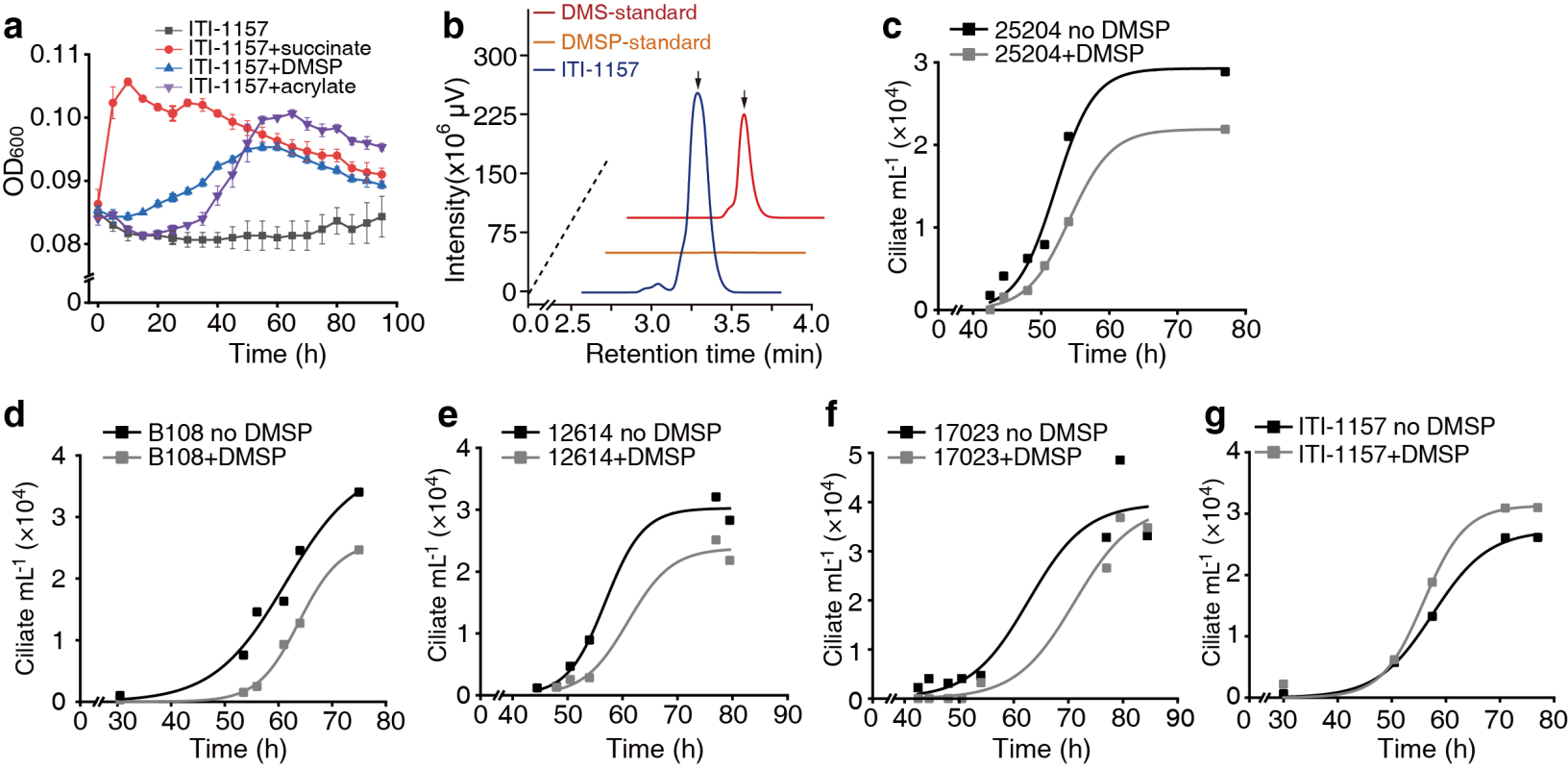

a
b
c
d
e
DMSP
No DMSP
***
***
20
10
0
1
2
3
Attracted cilitate
(Times/min)
Detained cilitate
(/min)
f
DMS
No DMS
**
**
30
20
10
0
1
2
3
Attracted ciliate
(Times/min)
Detained ciliate
(/min)
g
ΔdddL no DMS
ΔdddL+DMS
Ciliate mL-1 (×10 4)
Time (h)
h
SM1211 no acrylate
SM1211+acrylate
Acrylate
Ciliate mL-1 (×10 4)
Acrylate (μM)
Time (h)
i
ΔdddL no acrylate
ΔdddL+acrylate
Acrylate
Ciliate mL-1 (×10 4)
Acrylate (μM)
Time (h)
j
Ciliate no acrylate
Ciliate+70 μM acrylate
Ciliate+500 μM acrylate
Ciliate+1 mM acrylate
1 mM acrylate
500 μM acrylate
70 μM acrylate
Ciliate mL-1 (×10 4)
Acrylate (μM)
Time (h)

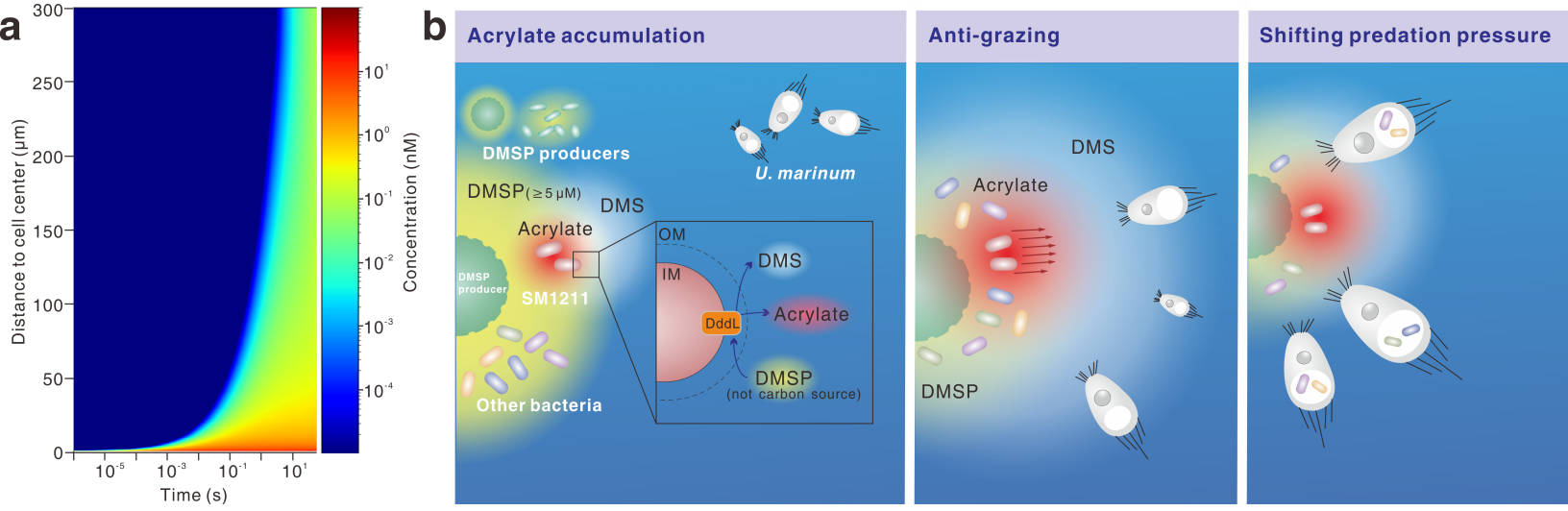

a
Without DMSP
SM1211
ΔdddL
DAPI
Overlay
With DMSP
SM1211
ΔdddL
DAPI
Overlay
b
No Acrylate
5 mM Acrylate
Log10 (CFU/mL)
Time (h)
c
P. arc
OD600
no acrylate
1 mM acrylate
5 mM acrylate
10 mM acrylate
Time (h)
d
Control
P. arc+succinate
P. arc+DMSP
P. arc+acrylate
OD600
Time (h)
e
Control
4-2+succinate
4-2+DMSP
4-2+acrylate
OD600
Time (h)
f
Control
K15+succinate
K15+DMSP
K15+acrylate
OD600
Time (h)
g
4-2
OD600
no acrylate
1 mM acrylate
5 mM acrylate
10 mM acrylate
Time (h)
h
K15
no acrylate
1 mM acrylate
5 mM acrylate
10 mM acrylate
OD600
Time (h)
i
Log10 (SM1211/K15)
Log10 (SM1211/4-2)
Log10 (SM1211/P. arc)
Log10 (ΔdddL/K15)
Log10 (ΔdddL/4-2)
Log10 (ΔdddL/P. arc)
0 h
72 h no DMSP
72 h DMSP
72 h no DMSP no ciliates
72 h DMSP no ciliates
Prey abundance ratio


a
90° W
0°
90° E
60° N
30° N
EQ
30° S
60° S
0.02
Actinobacteria
Deltaproteobacteria
Gammaproteobacteria
Alphaproteobacteria


b
Puniceibacterium antarcticum strain SM1211
Roseovarius indicus strain B108
Jannaschia pohangensis strain DSM 19073
Cribrihabitans marinus strain DSM 29340
Albimonas pacifica strain CGMCC 1.11030
Desulfonatronovibrio magnus strain AHT22
Phycicoccus sp. CMS6Z-2
Roseospirillum parvum strain 930I
Marinobacter similis strain A3d10
Salinisphaera orenii MK-B5
Sulfitobacter litoralis strain DSM 17584
Marivita cryptomonadis strain CL-SK44
Labrenzia aggregata strain RMAR6-6
Granulosicoccus antarcticus IMCC3135


c
Aquatic source
Host-associated
Biofilm
DddL detected
ND
Sediment
Lagoon
Saline water
Salt marsh
Cyanophyta
alga
Salt marsh grass
Spongia
Coral
Fish
Biofilm
0
100
200
400
Sample numbers


a
ITI-1157
ITI-1157+succinate
ITI-1157+DMSP
ITI-1157+acrylate
OD600
Time (h)
b
DMS-standard
DMSP-standard
ITI-1157
Intensity(x10^6 μV)
Retention time (min)
c
25204 no DMSP
25204+DMSP
Ciliate mL-1 (×10^4)
Time (h)
d
B108 no DMSP
B108+DMSP
Ciliate mL-1 (×10^4)
Time (h)
e
12614 no DMSP
12614+DMSP
Ciliate mL-1 (×10^4)
Time (h)
f
17023 no DMSP
17023+DMSP
Ciliate mL-1 (×10^4)
Time (h)
g
ITI-1157 no DMSP
ITI-1157+DMSP
Ciliate mL-1 (×10^4)
Time (h)


a
Distance to cell center (μm)
300
250
200
150
100
50
0
10^{-5}
10^{-3}
10^{-1}
10^{1}
Time (s)
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
Concentration (nM)
b
Acrylate accumulation
DMSP producers
U. marinum
DMSP(≥5 μM)
DMS
Acrylate
DMSP producer
SM1211
Other bacteria
OM
IM
DddL
DMS
Acrylate
DMSP
(not carbon source)
Anti-grazing
DMS
Acrylate
DMSP
Shifting predation pressure