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Supplementary Materials for

Bacterial virulence against an oceanic bloom-forming phytoplankter is mediated by algal DMSP

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Supplementary Text

Text S1. Coculturing of *E. huxleyi* with the CAM exhibits similar phases of pathogenicity to that of *Sulfitobacter* D7.

Time course of *E. huxleyi* cultures incubated with CAM (fig. S1C-E) showed three-phase dynamics, similar to co-culturing with *Sulfitobacter* D7 (Fig. 1B-D). In phase I, algal cultures grew exponentially until day 5, similar to control cultures (fig. S1C). In phase II, while control cultures kept growing, CAM-treated cultures entered a short 2-day stationary phase. During the 4 days of phase III, there was a rapid decline in algal abundance, while control cultures kept growing until they reached stationary phase. Induction of algal cell death (determined by SYTOX green staining) in phase III occurred in ~80% of the population (fig. S1D) and was concomitant with rapid exponential growth of bacteria (overall growth of four orders of magnitude) (fig. S1E). Interestingly, during phases II and III of co-culturing, we detected a similar scent to that emitted during incubation of *E. huxleyi* with *Sulfitobacter* D7 (fig. S1C-E, represented by green background). Interestingly, the abundance of *Sulfitobacter* D7 during co-culturing with CAM increased steadily by 3 orders of magnitude, as quantified by qPCR (fig. S1E, inset).

Text S2. *Sulfitobacter* D7 consumes DMSP and produces MeSH but not DMS.

In order to characterize the origin of MeSH production during *Sulfitobacter* D7-infection of *E. huxleyi* (Fig. 3), we obtained conditioned media (CM) derived from 0.22 µm filtrate of uninfected *E. huxleyi* cultures at stationary phase (*E. huxleyi*-CM). We inoculated *Sulfitobacter* D7 in *E. huxleyi*-CM and found that after 24h of incubation the concentration of $DMSP_d$ was 51 µM, while in uninoculated (blank) *E. huxleyi*-CM it was 72 µM (table S1). Namely, *Sulfitobacter* D7 consumed ~21 µM $DMSP_d$ from the medium concomitant to production of MeSH. In order to validate that the production of MeSH by *Sulfitobacter* D7 resulted from DMSP (rather than other substrates within *E. huxleyi*-CM), we inoculated *Sulfitobacter* D7 in minimal media (MM) supplemented with synthetic DMSP. Also here, *Sulfitobacter* D7 consumed $DMSP_d$ and produced MeSH but to a lesser extent than in *E. huxleyi*-CM (table S1). A similar observation was seen for the

bacterial growth. This implies that *E. huxleyi*-CM contains other substances that promote *Sulfitobacter* D7 growth and metabolism. Taken together, *Sulfitobacter* D7 can produce MeSH from $DMSP_d$ in MM and in *E. huxleyi*-CM and most likely during *Sulfitobacter* D7-infection of *E. huxleyi*. Interestingly, in both media the concentration of DMS was similar between uninoculated and inoculated media (table S1). Therefore, it seems that *Sulfitobacter* D7 does not produce DMS from DMSP. *Sulfitobacter* spp. have been reported to encode for DddL, a DMSP-lyase enzyme (*14*, *71*), however, we did not detect any homologs of a DMSP-lyase (*ddd* genes) in *Sulfitobacter* D7 genome.

Supplementary Figures

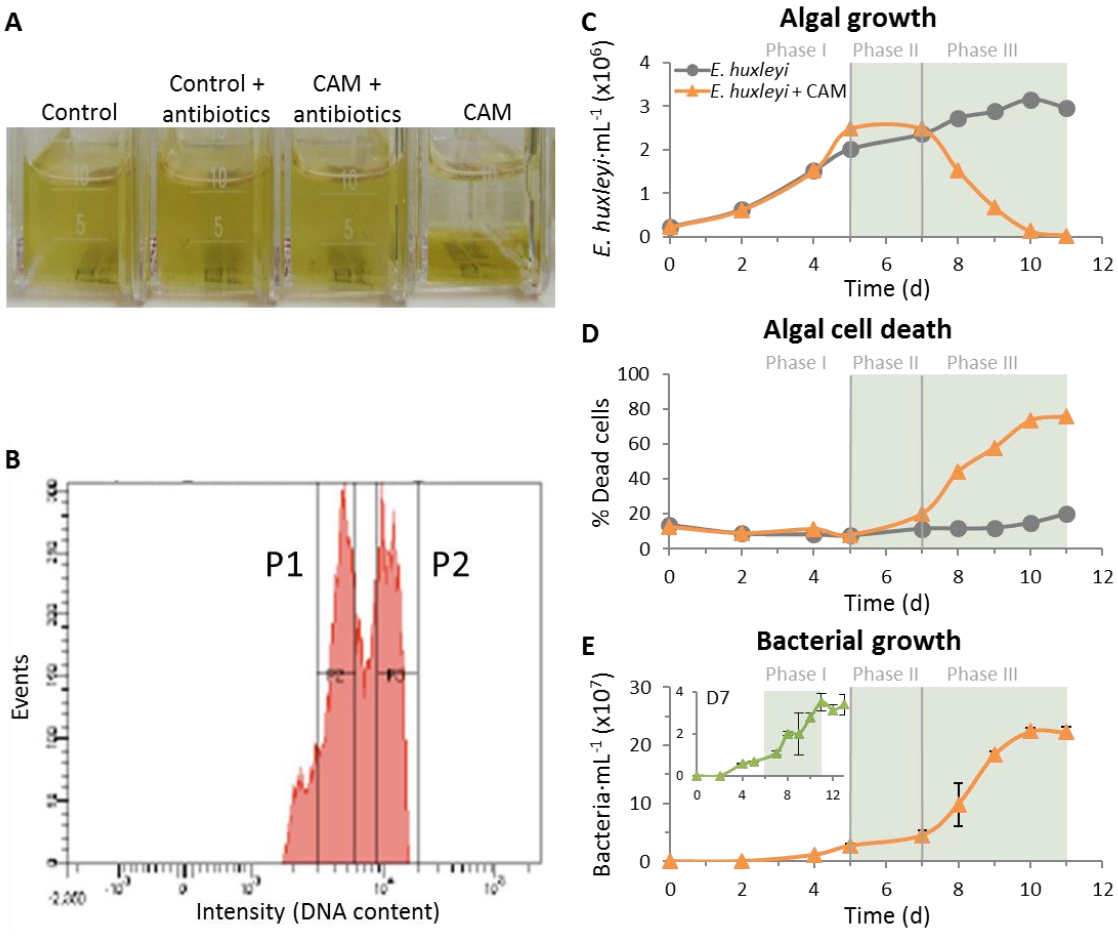

Fig. S1. Algicidal effect of the CAM on *E. huxleyi*. (**A**) Picture of *E. huxleyi* 379 cultures (control or incubated with CAM) applied (or not) with penicillin and streptomycin antibiotics mix. (**B**) Flow cytometric analysis of bacterial populations, stained with SYTO13, in *E. huxleyi* 379 cultures incubated with CAM after 7 days of growth. Bacteria were differentiated based on green fluorescence (530/30 nm) intensity (arbitrary units) corresponding to DNA content. Two bacterial sub-populations were sorted: P1 and P2, featured low and high green fluorescence intensity, respectively. *Sulfitobacter* D7 and *Marinobacter* D6 were each isolated from a single colony of P2 population plated on marine agar. (**C**-**E**) A detailed time course of *E. huxleyi* 379 mono-cultures (grey line) and during co-culturing with CAM (orange line). The following parameters were assessed: algal growth (C), algal cell death (D) and bacterial growth (E). Inset in (E): quantification of *Sulfitobacter* D7 abundance during co-culturing of *E.*

huxleyi with CAM, determined by qPCR analysis. No bacterial growth was observed in control cultures. Green background represents the presence of a pungent scent in co-cultures. Alga-bacteria co-culturing had distinct dynamics characterized by defined phases (I-III) of pathogenicity. Results depicted in (C-E) represent average ± SD (n = 3). Error bars < than symbol size are not shown. Statistical differences in (C-E) were tested using repeated measures ANOVA. *P*-values are <0.001 for the differences between control and CAM-treated *E. huxleyi* cultures.

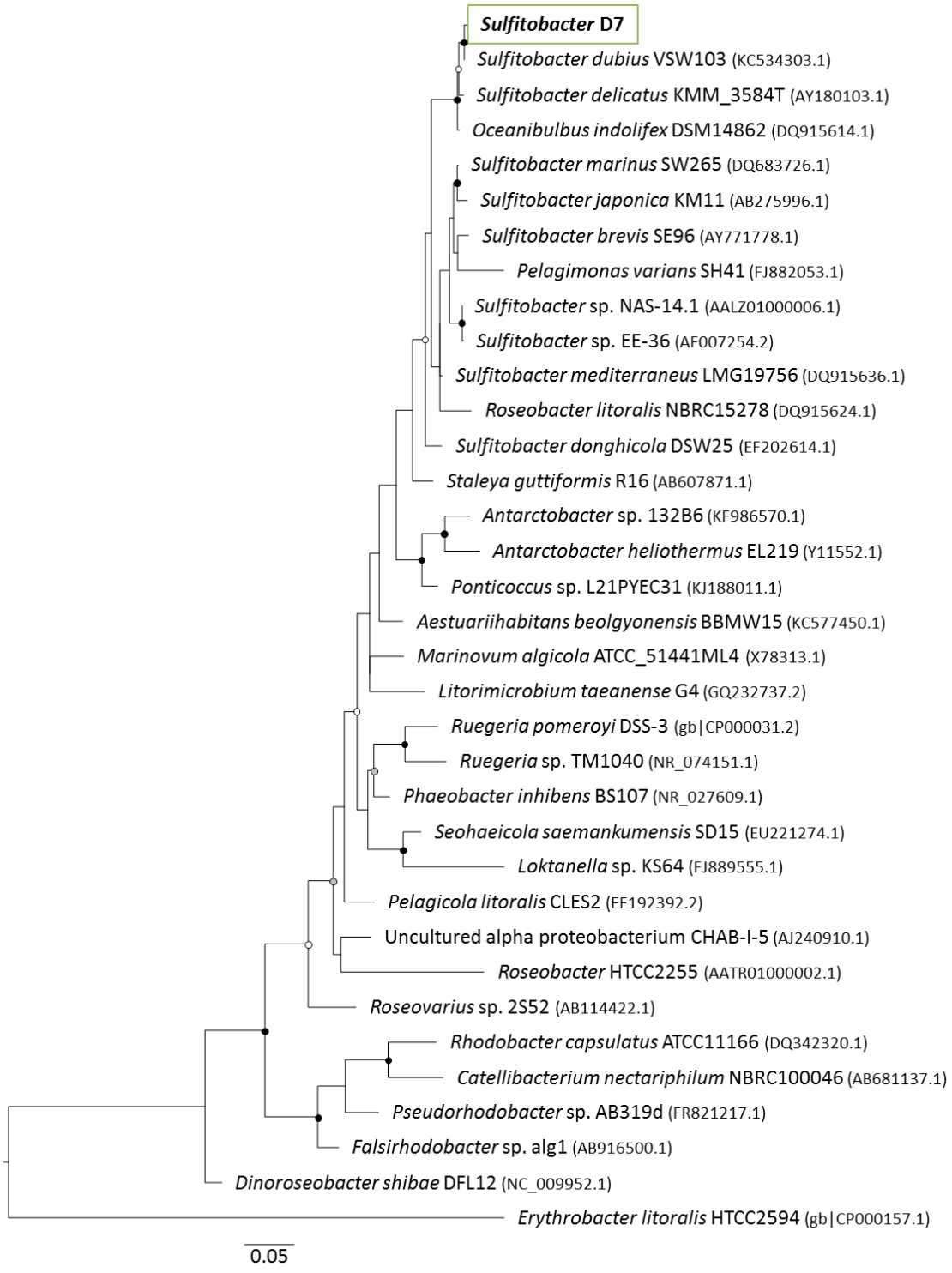

Fig. S2. Phylogenetic analysis of *Sulfitobacter* D7 within the *Roseobacter* group.
Maximum likelihood phylogenetic tree of the *Roseobacter* group of the α-Proteobacteria class, based on 16S rRNA gene. Bootstrap values (based on 1000 replicates) are specified

with circles at the nodes (white ≥ 50%, grey ≥ 70%, black ≥90%). *Sulfitobacter* D7 isolate can be found within the group (green box), closely associated with *Sulfitobacter dubius*. *Erythrobacter lithoralis*, affiliated to the α-Proteobacteria class, was used as an outgroup.

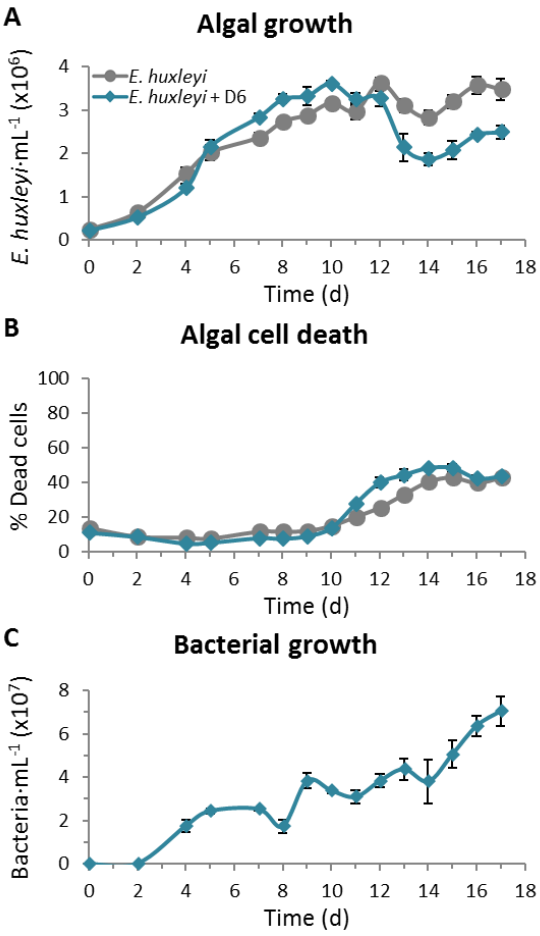

Fig. S3. *Marinobacter* D6 isolated from CAM has no algicidal effect when cocultured with *E. huxleyi*. A detailed time course of *E. huxleyi* 379 mono-cultures (grey line) and during co-culturing with *Marinobacter* D6 (blue line). The following parameters were assessed: (**A**) algal growth, (**B**) algal cell death and (**C**) bacterial growth. No bacterial growth was observed in control cultures. Results represent average ± SD (n = 3). Error bars < than symbol size are not shown. Statistical differences were tested using repeated measures ANOVA. *P*-values are <0.05 for the differences between control and D6-treated *E. huxleyi* cultures.

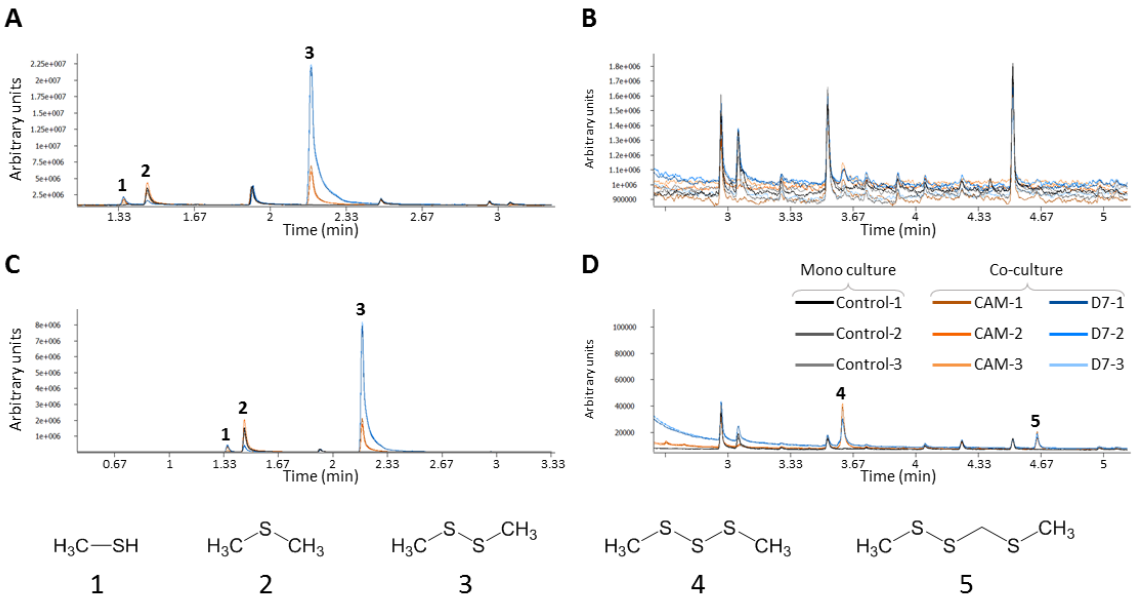

Fig. S4. Headspace analysis of volatiles produced during algae-bacteria interactions using SPME coupled to GC-MS. (**A-B**) Representative total ion chromatograms of headspaces of control, CAM- and *Sulfitobacter* D7-infected *E. huxleyi* 379 cultures at 10 days of growth (phase III). Detected compounds- 1: methanethiol (MeSH); 2: dimethyl sulfide (DMS); 3: dimethyl disulfide (DMDS), 4: dimethyl trisulfide (DMTS); 5: methyl methylthiomethyl disulfide. (**C-D**) Extracted ion chromatograms represent characteristic masses (47, 62, 94, 61, 126 *m/z*) of compounds 1-5, respectively. Analysis was done in triplicates.

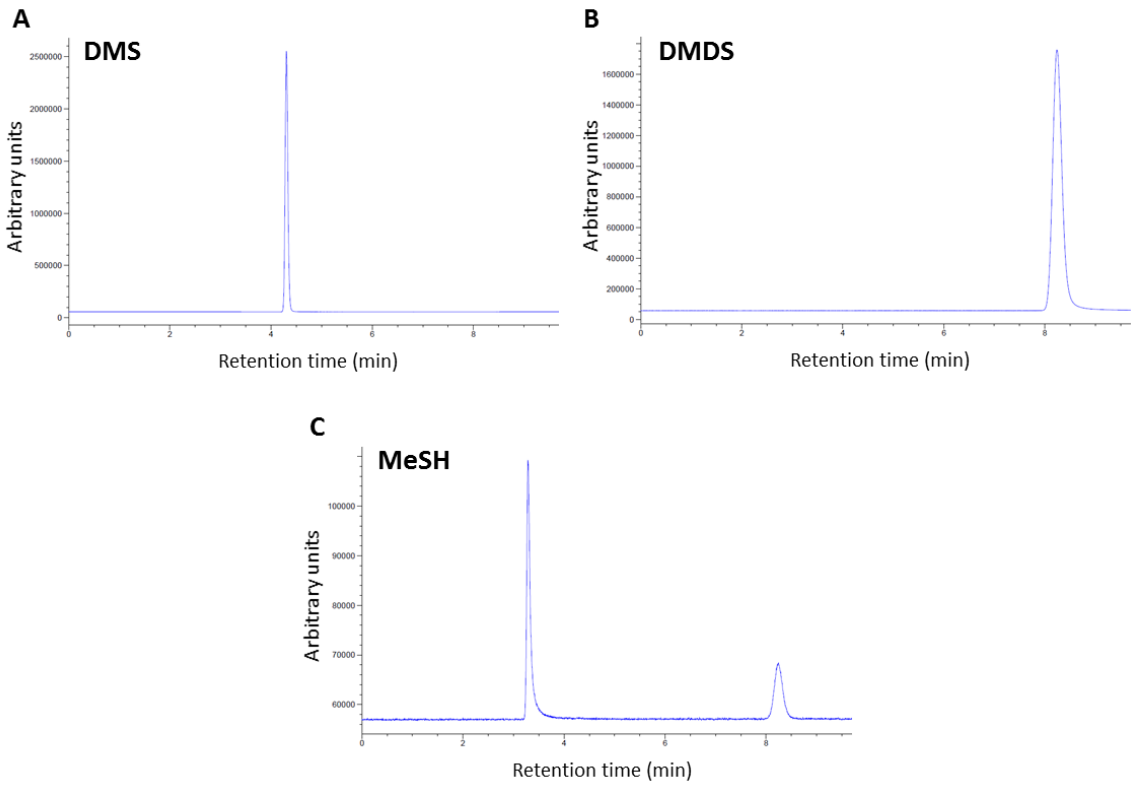

Fig. S5. Representative chromatograms of VOSC standards in GC-FPD analysis. Representative GC-FPD chromatograms of 150 nM of (**A**) dimethyl sulfide (DMS), retention time of 4.3 min; (**B**) dimethyl disulfide (DMDS), retention time of 8.2 min; and (**C**) methanethiol (MeSH), retention time of 3.3 min. The additional peak in (C) is DMDS which is a product of MeSH oxidation that occurred during the GC-FPD procedure (*39*).

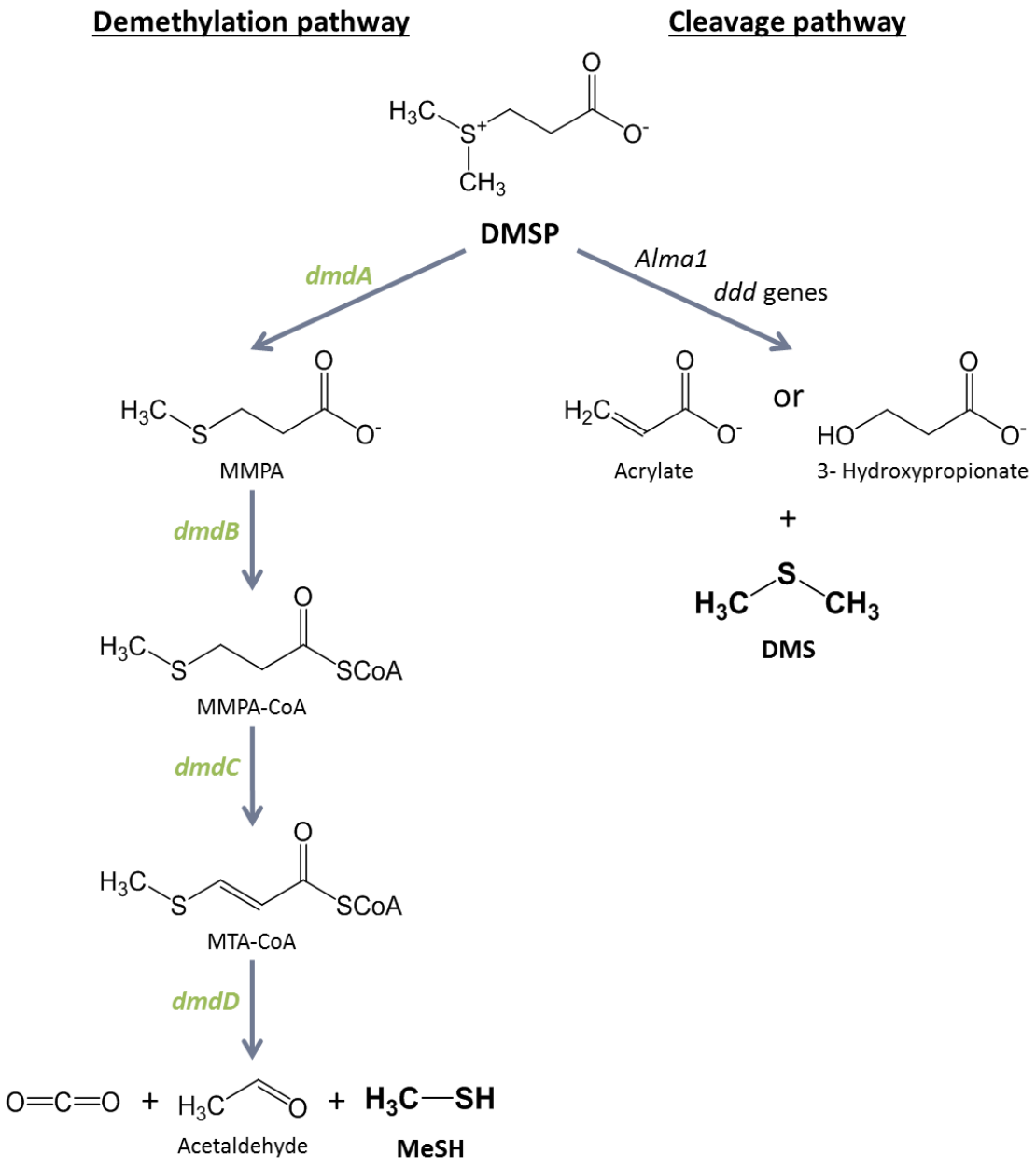

Fig. S6. *Sulfitobacter* D7 genome encodes a DMSP catabolic pathway. Competing DMSP catabolic pathways. Genes encoding enzymes mediating each transformation are specified next to the arrow. Genes of the demethylation pathway, highlighted in green, are present in *Sulfitobacter* D7 genome. MMPA, methylmercaptopropionate; CoA,

coenzyme A; MMPA-CoA, methylmercaptopropionate-CoA; MTA-CoA, methylthioacryloyl-CoA.

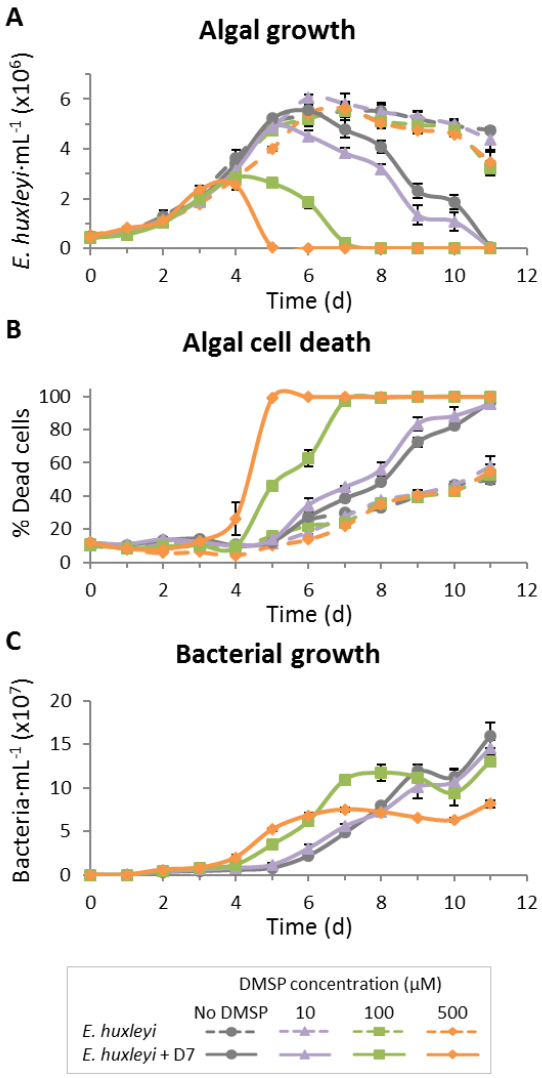

Fig. S7. DMSP promotes *Sulfitobacter* D7 virulence toward *E. huxleyi* in a dose-dependent manner. Time course of *E. huxleyi* 379 mono-cultures (dashed lines) and during co-culturing with *Sulfitobacter* D7 (smooth lines). DMSP was applied at day 0 to a final concentration of 10 µM (purple, triangle), 100 µM (green, square), 500 µM (orange, diamond) or none (gray, circle). The following parameters were assessed: (**A**) algal growth, (**B**) algal cell death and (**C**) bacterial growth. No bacterial growth was observed in control cultures. Results represent average ± SD (n = 3). Error bars < than symbol size are not shown. Statistical differences were tested using two-way repeated measures ANOVA, accounting for infection and DMSP concentration. *P*-values in (A) and (B) are <0.001 for the differences between control and co-cultures and for the

differences between the DMSP treatments in co-cultures. *P*-values in (C) are <0.001 for the differences between the 100 µM DMSP treatment and the rest of the co-cultures.

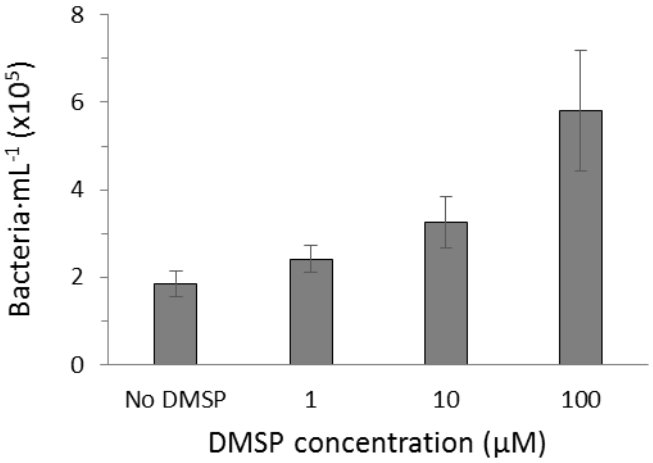

Fig. S8. DMSP promotes growth of *Sulfitobacter* D7. *Sulfitobacter* D7 abundance after 16 h of growth in minimal media (MM) supplemented with different concentrations of DMSP. Results represent average ± SD (n = 3). Statistical differences were tested using one-way ANOVA, followed by a Tukey post-hoc test. *P*-values are <0.01 and <0.05 for the differences of the 100 µM concentration from the "No DMSP" and 1 µM concentration, respectively.

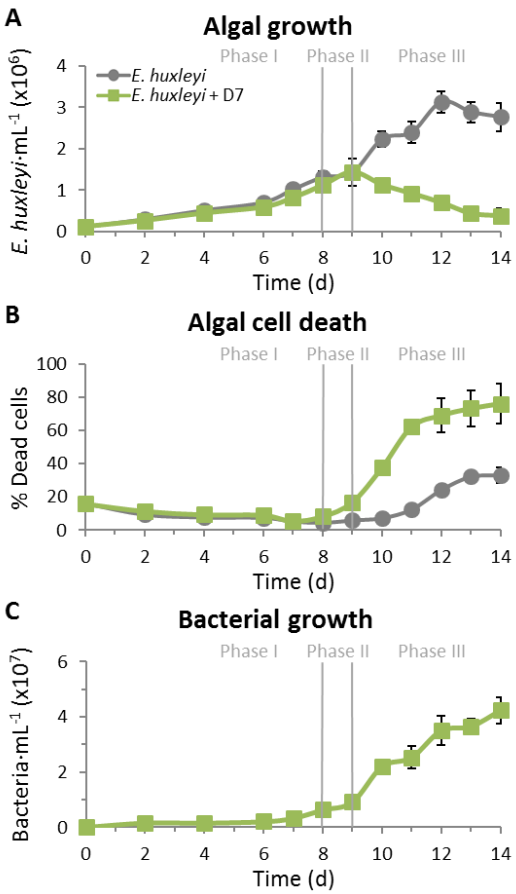

Fig. S9. *E. huxleyi* and *Sulfitobacter* D7 coculturing dynamics. Time course of *E. huxleyi* 379 mono-cultures (grey line) and during co-culturing with *Sulfitobacter* D7 (green line) from the experiment presented in Fig. 3. The following parameters were assessed: (**A**) algal growth, (**B**) algal cell death and (**C**) bacterial growth. No bacterial growth was observed in control cultures. Defined phases (I-III) of pathogenicity are denoted. Results represent average ± SD (control, n = 4; *Sulfitobacter* D7-infected, n = 2). Error bars < than symbol size are not shown. Statistical differences were tested using repeated measures ANOVA. *P*-values are <0.001 for the differences between control and co-cultures.

Supplementary Tables

Table S1. Evaluation of $DMSP_d$, MeSH, DMDS, DMS, and bacterial abundances after 24-hour incubation of *Sulfitobacter* D7 in CM obtained from uninfected *E. huxleyi* 379 cultures (*E. huxleyi*–CM) or MM supplemented with DMSP.

		$DMSP_d$ (µM)	MeSH[a]	DMDS (nM)[b]	DMS (nM)	*Sulfitobacter* D7 abundance ($10^6 \cdot mL^{-1}$)
E. huxleyi-CM	- D7[c]	72	–	–	406	–
	+ D7[d]	51 ± 0.3	1017 ± 34	465 ± 11	398 ± 24	94.4 ± 0.5
MM + DMSP	- D7[c]	70	–	–	23	–
	+ D7[f]	65 ± 1	46 ± 33	276 ± 19	16 ± 1.5	10.14 ± 0.97

[a] Square root of peak area
[b] DMDS is presumably an oxidation product of MeSH (Fig. S5c) and therefore considered as part of the MeSH pool
[c] Blank media without *Sulfitobacter* D7 (n = 1)
[d,f] Results represent average ± SD ([d]n = 2, [f]n = 3)
P-values were calculated for all parameters between *Sulfitobacter* D7-inoculated media and are <0.01

Table S2. Comparison of parameters related to *Sulfitobacter* D7 infection dynamics in various *E. huxleyi* strains.

	***Emiliania huxleyi* strain**			
	379	1216	373	2090
$DMSP_d$ (µM) at stationary growth[a]	71.9 ± 1.8	27.1 ± 0.4	13.2 ± 0.8	5.5 ± 0.1
Duration of phase III (days)[b]	5	7	10	–
Duration of phase II (days)	1	4	2	16
Sulfitobacter D7 abundance on phase III initiation ($10^7 \cdot mL^{-1}$)	1.28 ± 0.5	2.9 ± 0.3	1.28 ± 0.2	–
Maximum *Sulfitobacter* D7 abundance during co-culturing ($10^7 \cdot mL^{-1}$)[c]	15 ± 0.3	12 ± 0.7	15 ± 0.4	1.5 ± 0.6
Sulfitobacter D7 abundance after 24h growth in *E. huxleyi*-CM ($10^7 \cdot mL^{-1}$)[d]	12 ± 0.7	7.5 ± 0.2	2 ± 0.2	0.8 ± 0.2

Results represent average ± SD (n = 3)
[a] At 11 days of mono-culture (Fig. 4A)
[b] Until *E. huxleyi* cultures reached <1% of maximum growth (Fig. 4C-F)
[c] For 2090 at t = 20d, other strains at t = 21d (Fig. 4C-F)
[d] Conditioned media (CM) was derived from the same cultures presented in the first row of this table