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ORIGINAL ARTICLE

A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals

Melissa Garren[1], Kwangmin Son[2], Jean-Baptiste Raina[3,4], Roberto Rusconi[1], Filippo Menolascina[1], Orr H Shapiro[1,5], Jessica Tout[6], David G Bourne[3], Justin R Seymour[6] and Roman Stocker[1]

[1]*Ralph M Parsons Laboratory, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA;* [2]*Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA;* [3]*Australian Institute of Marine Science, PMB3, Townsville, QLD, Australia;* [4]*AIMS@JCU, ARC Centre of Excellence for Coral Reef Studies and School of Marine and Tropical Biology, James Cook University, Townsville, QLD, Australia;* [5]*Department of Plant Sciences, Weizmann Institute of Science, Rehovot, Israel and* [6]*Plant Functional Biology & Climate Change Cluster (C3), University of Technology, Sydney, NSW, Australia*

Diseases are an emerging threat to ocean ecosystems. Coral reefs, in particular, are experiencing a worldwide decline because of disease and bleaching, which have been exacerbated by rising seawater temperatures. Yet, the ecological mechanisms behind most coral diseases remain unidentified. Here, we demonstrate that a coral pathogen, *Vibrio coralliilyticus*, uses chemotaxis and chemokinesis to target the mucus of its coral host, *Pocillopora damicornis*. A primary driver of this response is the host metabolite dimethylsulfoniopropionate (DMSP), a key element in the global sulfur cycle and a potent foraging cue throughout the marine food web. Coral mucus is rich in DMSP, and we found that DMSP alone elicits chemotactic responses of comparable intensity to whole mucus. Furthermore, in heat-stressed coral fragments, DMSP concentrations increased fivefold and the pathogen's chemotactic response was correspondingly enhanced. Intriguingly, despite being a rich source of carbon and sulfur, DMSP is not metabolized by the pathogen, suggesting that it is used purely as an infochemical for host location. These results reveal a new role for DMSP in coral disease, demonstrate the importance of chemical signaling and swimming behavior in the recruitment of pathogens to corals and highlight the impact of increased seawater temperatures on disease pathways.
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Introduction

The globally distributed marine bacterium *Vibrio coralliilyticus* (Pollock *et al.*, 2010) causes bleaching and tissue loss in reef-building corals (Ben-Haim *et al.*, 2003). Despite the widespread loss of corals to diseases (Harvell *et al.*, 2009), little is known about their onset, and fundamental questions, such as how a pathogen finds its host, have remained largely unanswered (Bourne *et al.*, 2009). Among human enteric pathogens, the ability to swim (motility) and guide movement in response to chemical gradients (chemotaxis) is a common phenotype in the infection process (Boin *et al.*, 2004; Croxen *et al.*, 2006). In the ocean, we found that motility is universal among putative coral pathogens (Supplementary Table S1). This prevalence, together with the presence of strong chemical gradients that can extend over 2 mm from the coral surface (Kühl *et al.*, 1995; Mass *et al.*, 2010), suggests that motile responses to chemical cues may be a pervasive mechanism for coral pathogens to locate and colonize their hosts. Yet, beyond evidence that motility and chemotaxis are involved in *Vibrio*-induced bleaching (Banin *et al.*, 2001; Meron *et al.*, 2009), there has been no direct, real-time observation of the motile behavior of pathogens, nor any insight into the specific chemical triggers of chemotaxis or its dependence on the host's physiological state. By integrating microfluidic experiments with the collection of coral exudates, we found that

Correspondence: M Garren or R Stocker, Ralph M Parsons Laboratory, Department of Civil & Environmental Engineering, Massachusetts Institute of Technology, 15 Vassar Street, Cambridge, MA 02139, USA.
E-mail: mgarren@mit.edu or romans@mit.edu
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V. coralliilyticus (Pollock *et al.*, 2010) markedly changes its motility behavior in response to the mucus of its host, *Pocillopora damicornis*, to rapidly target the source of the cue.

The surface of a coral is lined with mucus of variable viscosity, which is continuously excreted for cleansing, feeding and defense (Brown and Bythell, 2005). This mucus contains a broad range of chemicals, including water-soluble glycoproteins, amino acids and metabolites (Brown and Bythell, 2005). In the mucus of many coral species, the sulfur compound dimethylsulfoniopropionate (DMSP) reaches concentrations (1–62 μM) orders of a magnitude higher than in the surrounding seawater (6–11 nM) (Broadbent and Jones, 2004; Van Alstyne *et al.*, 2006). For corals, this molecule might act as an antioxidant (Sunda *et al.*, 2002) or as an overflow system for the symbiotic zooxanthellae to excrete excess sulfur (Stefels, 2000). DMSP has also been shown to be a potent chemoattractant for several marine micro- and macro-organisms (Debose and Nevitt, 2007; Seymour *et al.*, 2010). Here, we show that DMSP is a primary chemical cue for *V. coralliilyticus*' behavioral responses to the mucus of *P. damicornis* and that its increased production under heat stress enhances the attraction of the pathogen.

Materials and methods

Organism growth conditions and laboratory mucus collection

All experiments were conducted using *V. coralliilyticus*, strain BAA-450, acquired from the American Type Culture Collection (www.atcc.org, Manassas, VA, USA) and grown in 0.2 μM filtered, autoclaved seawater (FASW) with 1% 2216 media (BD Difco) in a shaking incubator at 30°C. Small colonies of the coral *P. damicornis* (from the Birch Aquarium at Scripps, La Jolla, CA, USA) were cultured at 25 °C in artificial seawater (Instant Ocean, Spectrum Brands Company, Cincinnati, OH, USA) on a 12-h light–dark cycle. Mucus was collected from the colonies by exposing them to air for 3 min. Owing to volume requirements of the microfluidic assays, the mucus was then diluted to 1:2 in FASW and vortexed for 10 s to mix thoroughly.

Mucus collection on Davies Reef (Great Barrier Reef)

Small colonies of the coral *P. damicornis* and *Acropora millepora* were collected from Davies Reef, Great Barrier Reef, Australia (18°05′ S/147°39′ E) and transferred to the outdoor aquarium facility of the Australian Institute of Marine Science (Townsville, QLD, Australia). Mucus was collected from the colonies by removing them from the water, shaking off excess water for 10 s and then holding them upside down collecting dripping mucus with a syringe. Freshly collected mucus was then homogenized and divided in two: one half was flash-frozen in liquid nitrogen; the second half was directly extracted with 40 ml of high-performance liquid chromatography-grade methanol for DMSP quantification. The frozen portion was later used in chemotaxis assays.

Metabolism and DMSP measurements

DMSP metabolism. Two different basal media were used to determine the DMSP metabolic capabilities of *V. coralliilyticus*: a modified marine ammonium salt medium (Raina *et al.*, 2009) lacking any carbon source, and a modified basal salt medium lacking any sulfur source (Fuse *et al.*, 2000). DMSP was added to both the media (1 mM final concentration) and acted either as a sole carbon source or as a sole sulfur source. The pH was adjusted to 8.2. To account for the potential cometabolism of DMSP with other compounds present in coral mucus, mucus was collected as described above, homogenized, filtered twice (0.2 μm) and sonicated for 10 min. Five milliliters of marine ammonium salt medium, modified basal salt medium or sterile mucus were inoculated in triplicate from single *V. coralliilyticus* colonies and incubated at 28 °C between 1 and 6 days with shaking in gas-tight vials. Control bottles containing only the basal media and DMSP were used to account for the possible chemical breakdown of DMSP. Results from these experiments were confirmed using an alternative *V. coralliilyticus* strain, LMG 23696 (Sussman *et al.*, 2008). Bottles inoculated with the *Pseudovibrio* sp. P12 (an alphaproteobacterium isolated from healthy *P. damicornis*) grown under identical conditions acted as the positive control.

Acrylate metabolism. Marine ammonium salt medium lacking carbon was used to investigate the ability of strain BAA-450 to degrade acrylate (1 mM, final concentration). Five milliliters of marine ammonium salt medium were inoculated in triplicate from single BAA-450 colonies and incubated at 28 °C between 1 and 6 days with shaking. Control bottles containing only the basal medium and acrylate were set up, along with the ones inoculated with BAA-450, to account for its possible chemical breakdown. Bottles inoculated with the *Pseudovibrio* sp. P12 grown under identical conditions served as a positive control.

NMR measurements. DMSP metabolism assays and DMSP quantification were performed by ^{1}H nuclear magnetic resonance (NMR) (Tapiolas *et al.*, 2013). Briefly, the headspace of each gas-tight vial was first sampled with a syringe. Methanol (40 ml) was then added to each culture tube to extract DMSP and acrylate, and the mixtures were subsequently dried *in vacuo* using a rotary evaporator (Buchi, Flawil, Switzerland). The dried extracts were resuspended in a mixture of deuterated methanol (CD_3OD, D 99.8%, 750 μl) and deuterium oxide (D_2O, D 99.8%, 250 μl) (Cambridge Isotope

Laboratories, Andover, MA, USA). A 750-µl aliquot of the particulate-free extract was transferred into a 5-mm Norell tube (Norell Inc., Landisville, NJ, USA) and analyzed immediately by ^{1}H NMR. Spectra were recorded on a Bruker Avance 600 MHz NMR spectrometer (Billerica, MA, USA) with a TXI 5 mm probe and quantification was performed using the Electronic REference To access *In vivo* Concentrations method (ERETIC) (Tapiolas *et al.*, 2013). No DMSP degradation, acrylate by-products or dimethylsulfide smell were present in the DMSP medium experiments for *V. coralliilyticus* or the negative control, whereas all were present in the *Pseudovibrio* positive control. In the acrylate medium experiments, acrylate was degraded by both *V. coralliilyticus* and the *Pseudovibrio* positive control, but not the no-bacteria negative control. The same NMR protocol was also used to quantify the amount of DMSP present in coral mucus from *P. damicornis* and *A. millepora*.

Chemotactic index (I_C)

We quantified the magnitude of the chemotactic response using the chemotactic index, I_C, which measures the enhancement in the cell concentration within the region initially occupied by the mucus (that is, the central band in Supplementary Figure S1), relative to the cell concentration outside that area, minus 1. $I_C = 0$ thus corresponds to a uniform cell distribution (that is, no chemotaxis). See Seymour *et al.* (2010) for more details. For each experiment, triplicate 0.2 µm FASW control trials were run first, wherein the same FASW used to grow the cells and to make the DMSP dilutions was injected into the microfluidic device in lieu of an attractant. All I_C curves for a given attractant were normalized to their FASW control by subtracting the mean I_C among the three FASW trials.

To compare the I_C values observed in this study with values observed by Stocker *et al.* (2008) for *Escherichia coli* and *Pseudoalteromonas haloplanktis* in a similar (but not identical) experimental setup, data were extrapolated from Figure 2b of that manuscript and converted from the hot spot index (H) to I_C. The hotspot index was defined by Stocker *et al.* as the mean concentration of bacteria within the central, $W_C = 300$ µm wide region of the microchannel relative to the mean concentration over the entire channel width, $W = 1200$ µm. The data were converted to I_C using the following conversion formula: $I_C = ((W - W_C)/((W/H) - W_C)) - 1$.

Diffusive gradient microfluidic experiments

Microinjector device for chemotaxis assays. A 2.8-mm wide microchannel with a 400-µm wide injector (Supplementary Figure S1) was fabricated using soft lithography techniques described previously (Seymour *et al.*, 2008) to establish diffusive gradients for chemotaxis assays. Briefly, the attractant was injected into the microchannel (Supplementary Figure S1; inlet B) as a 400-µm wide band equidistant from the channel's side walls, whereas the cells were injected in the channel on either side of the band (Supplementary Figure S1; inlet A). The cells and attractant were flowed into the channel and then the flow was stopped to allow the attractant to diffuse laterally and the cells to respond to the gradient. DMSP (DMSP · HCl; C5H10SO2 · HCl; TCI) was freshly prepared with FASW to make 15 µM, 45 µM and 61 µM working solutions that closely corresponded to the amount of DMSP measured in the *P. damicornis* and *A. millepora* mucus samples. *A. millepora* was chosen as a second species to test because *V. coralliilyticus* is known to infect it as well (Sussman *et al.*, 2009). These freshly prepared DMSP solutions as well as *P. damicornis* mucus collected from Davies Reef (Great Barrier Reef; preserved at −80 °C (as described in the mucus collection section) and thawed on ice directly before experimental use; measured to contain 12–15 µM DMSP) and from corals maintained in the laboratory at MIT, *A. millepora* mucus from the Great Barrier Reef (containing 45–62 µM DMSP) and a FASW control were tested against overnight cultures of *V. coralliilyticus*.

The channel was loaded at moderate flow rates (2 µl min^{-1}) to establish an initial experimental condition where the cells and the attractant were in discrete bands (Supplementary Figure S1B). To begin the experiment, the flow was stopped and the channel was imaged directly downstream of the end of the microinjector using phase-contrast video microscopy on a Nikon Ti microscope (Tokyo, Japan) equipped with an Andor Neo CCD camera (6.5 µm/pixel; Belfast, UK) at 1 frame per second for 6 min. Five replicates of each experiment were conducted, and the microchannel was flushed for 30 s with fresh cells and attractant between replicates. Flushing with FASW lasted for 2 min in between different attractants. Each video was analyzed for cell positions using an automated image segmentation software developed in-house with MATLAB (MathWorks, Natick, MA, USA). Background subtraction and cross-correlation functions were used to detect non-motile cells or other particles from the mucus, which were excluded from the cumulative cell distribution across the channel. The resulting time series of cell distributions are presented for *P. damicornis* (Figure 1) and for *A. millepora* (Supplementary Figure S2).

Temperature stress experiment on Heron Island

To test the response of *V. coralliilyticus* to mucus from corals under high-temperature stress, a field experiment was carried out on Heron Island, Great Barrier Reef, Australia (23°26′ 37″S/151°54′ 44″E). Three colonies of *P. damicornis* were collected from the reef flat in front of the Heron Island Research

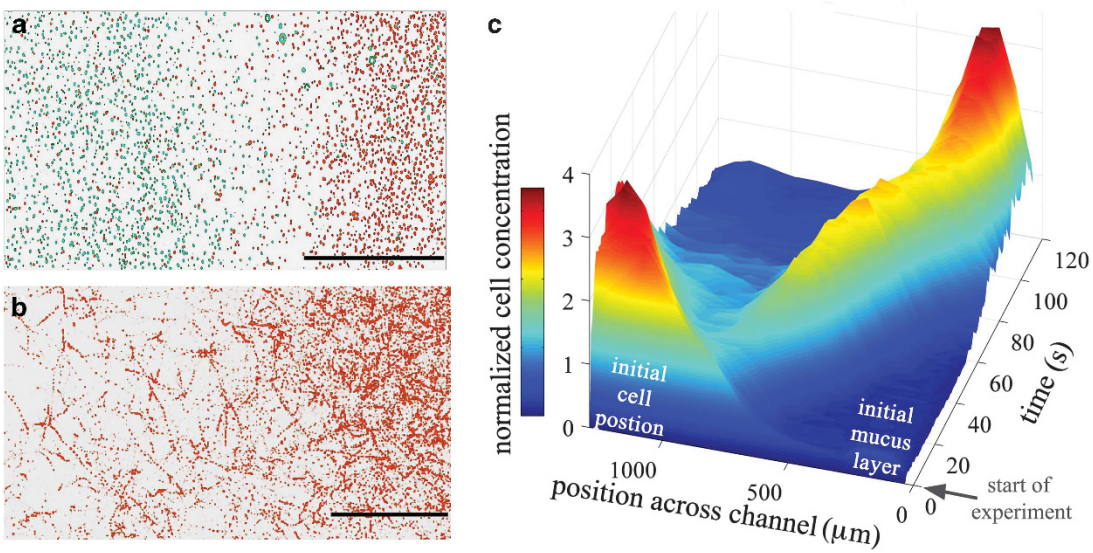

Figure 1 *V. coralliilyticus* is strongly attracted to coral mucus. (**a**) Positions and (**b**) trajectories of individual *V. coralliilyticus* cells exposed to a diffusing coral mucus gradient in a microfluidic channel (Supplementary Figure S1). A 400-μm thick layer of mucus, harvested from laboratory-cultured *P. damicornis* corals, was created in a microchannel (half of the layer is shown) and allowed to diffuse. The scale bars are 200 μm. In (**a**), cell positions at the start of the experiment and after two minutes are colored teal and red, respectively, and overlaid. In (**b**), trajectories acquired between 100 and 115 s after the start of the experiment are shown. The two panels show the strong shift in the cells' position and their intense accumulation into the mucus layer (the right side of the images). Also see Supplementary Movie S1. (**c**) The full time series of the spatial distribution of the pathogen population across the width of the microfluidic channel. Color and height both measure the local, instantaneous concentration of bacteria, normalized to a mean of one. Note the intense wave of bacteria actively migrating into the mucus layer.

Station, fragmented into 48 nubbins and allowed to recover and acclimate in a flow-through seawater tank pulling water from the reef flat for 8 days. Fragments were then distributed evenly into six tanks with three fragments from each donor colony in each tank. A randomized sample design for both fragment placement within the tanks and treatment assignment to each tank was employed. Three tanks were maintained at ambient seawater temperature (22 °C) for the duration of the experiment, and the other three tanks began at ambient temperature and then were slowly ramped by 1.5 °C per day for 7 days. All fragments were sampled for mucus by air exposure as described above at the initial time and after 7 days, when the temperature-treated tanks reached 31 °C. One-third of the mucus samples were preserved for DMSP measurements (described above) by adding 600 μl of methanol and freezing at −20 °C. Clonal replication is essential for comparing responses because DMSP concentration can vary with irradiance, zooxanthellae density and seawater temperature (Sunda *et al.*, 2002; Van Alstyne *et al.*, 2006). The rest of the samples were immediately frozen at −80 °C unaltered and shipped to MIT, where they were used in microfluidic chemotaxis experiments with the microinjector setup (Supplementary Figure S1). Replicate mucus samples from the heat-stress experiment were tested on three different days in the lab with freshly grown *V. coralliilyticus* cells. All trials yielded comparable results to those shown in the main text (Figure 2b; Supplementary Figure S6).

Mathematical model of simultaneous chemotaxis and chemokinesis

We modeled the chemotaxis and chemokinesis of *V. coralliilyticus* using an existing modeling framework for bacterial chemotaxis (Brown and Berg, 1974; Jackson, 1987; Kiørboe and Jackson, 2001), augmented by a concentration-dependent swimming speed that was based on our experimental observations (Figure 3a; Supplementary Information; Supplementary Figures S4 and S9). For simplicity and based on results from Figure 3a, we modeled chemokinesis as a 24% increase in swimming speed, from 66 μm s^{-1} at a relative chemoattractant concentrations of $C \leqslant 20\%$ of pure attractant to 82 μm s^{-1} for $C > 20\%$. From the spatial distribution of 3000 cells across the channel at each time point, we computed the time series of I_C (as detailed in the chemotactic index section of Materials and Methods). This was done twice: once in the presence of chemokinesis, and once in the absence of chemokinesis, in which case the swimming speed was uniformly equal to 66 μm s^{-1}. Results are presented in Figure 3b.

Results and discussion

To examine the ecological mechanism behind coral infection by *V. coralliilyticus*, we performed chemotaxis experiments using a microfluidic assay. *V. coralliilyticus* responded to coral mucus with remarkable speed and directionality. A microfluidic

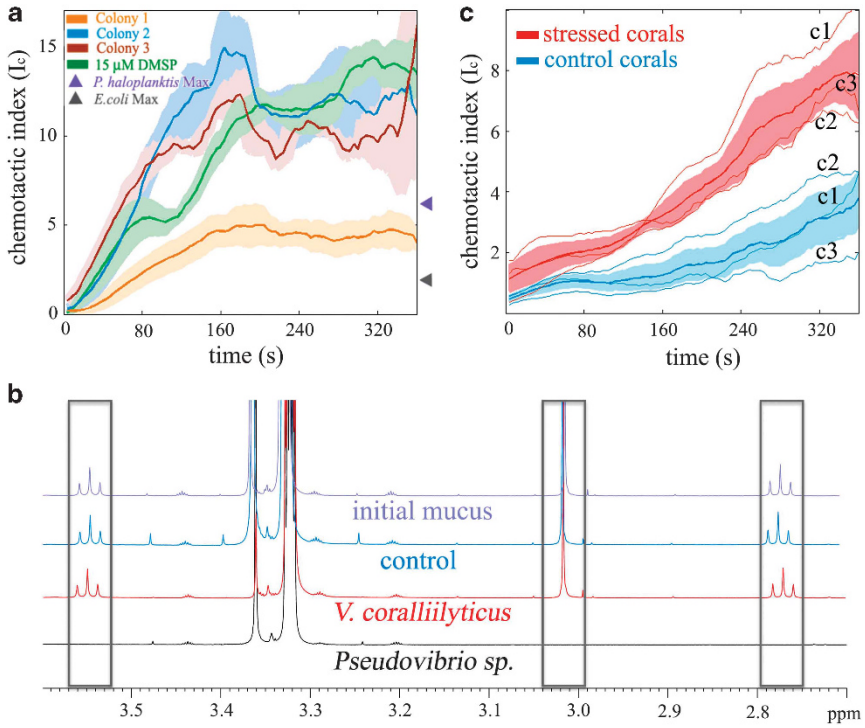

Figure 2 The pathogen's chemotaxis is primarily triggered by DMSP and is enhanced by heat stress of the host. (**a**) Time series of the chemotactic index, I_C, (a measure of the strength of cell accumulation) of *V. coralliilyticus* in response to a 400-µm thick layer (Supplementary Figure S1) of coral mucus (Colonies 1–3) or 15 µM DMSP (green line). Solid lines and shading represent the mean and s.e. of three replicate experiments. Mucus was collected from three different colonies of *P. damicornis* on Heron Island and contained 11.9–14.8 µM DMSP (Supplementary Information). The pathogen responds with comparable intensity to DMSP and mucus. Shown for reference are also the maximum chemotactic indices (suitably converted from Stocker *et al.* (2008) attained over 15 min by *E. coli* responding to a mixture of two of its most potent chemoattractants at near-optimal concentrations (serine and aspartate, 10 µM each; gray triangle) and by *P. haloplanktis* responding to algal exudates (purple triangle). All data were normalized against the respective no-attractant controls. (**b**) Profiles from quantitative NMR of freshly collected *P. damicornis* mucus from Heron Island (initial mucus, purple) reveal distinct peaks for DMSP (gray boxes). Twenty-four hours of incubation of whole mucus with *V. coralliilyticus* (red) resulted in no measurable DMSP degradation, akin to the no-bacteria control (blue), whereas the positive control strain *Pseudovibrio spp.* degraded DMSP entirely, as evidenced by the disappearance of the DMSP peaks (black). (**c**) Time series of *V. coralliilyticus*' I_C, in response to coral mucus from a clonally replicated temperature-stress experiment (maximum = 31 °C) performed on Heron Island. Chemotaxis was twice as strong toward mucus from stressed coral fragments (red) as compared with fragments from the same colonies maintained at ambient temperature (blue). Thin lines show each of the three individual colonies (c1–c3), bold lines show their mean and shading represents the s.e. All curves were normalized against seawater controls.

device was used to create a 400-µm thick layer of mucus adjacent to a 1-mm thick seawater suspension of *V. coralliilyticus*, and we imaged the spatial distribution of the pathogen population with high-temporal-resolution (15 frames s^{-1}) video microscopy (Figure 1; Supplementary Figure S1). Within 10 s of exposure to the mucus, bacteria began swimming up the associated chemical gradient. Within 60 s, >50% of cells had migrated into the 400-µm thick layer of mucus (Figure 1; Supplementary Movie S1). We quantified the magnitude of the pathogen's chemotactic response with the chemotactic index, I_C (Seymour *et al.*, 2010), which measures the enhancement in cell concentration within the initial mucus layer relative to the cell concentration outside that layer ($I_C = 0$ corresponds to no chemotaxis). *V. coralliilyticus* reached $I_C > 14$ within 3 min of being exposed to mucus (Figure 2a), a much more intense response than previously observed for either enteric or marine bacteria (Figure 2a). The strength of this response was confirmed by tracking individual bacteria and quantifying their mean chemotactic velocity (Supplementary Figures S3–S5), which reached 36% of the average swimming speed, considerably higher than the 5–15% typical of the model organism for bacterial chemotaxis, *E. coli* (Ahmed and Stocker, 2008).

To determine the chemical signal responsible for this response, we analyzed coral mucus using quantitative NMR (Tapiolas *et al.*, 2013). The DMSP concentrations in mucus from healthy colonies collected on Davies Reef, were high, ranging from 11.9–14.8 (±1.2) µM for *P. damicornis* and up to 62.2 (±2.0) µM for *A. millepora*, another coral species susceptible to *V. coralliilyticus* infection (Sussman *et al.*, 2009). Additional chemotaxis experiments revealed that DMSP (15 µM), when used as the sole attractant, elicited a chemotactic response of comparable magnitude to *P. damicornis* mucus ($I_{C,MAX} \sim 14$; Figure 2a). The pathogen's response varied somewhat from colony to colony, as is

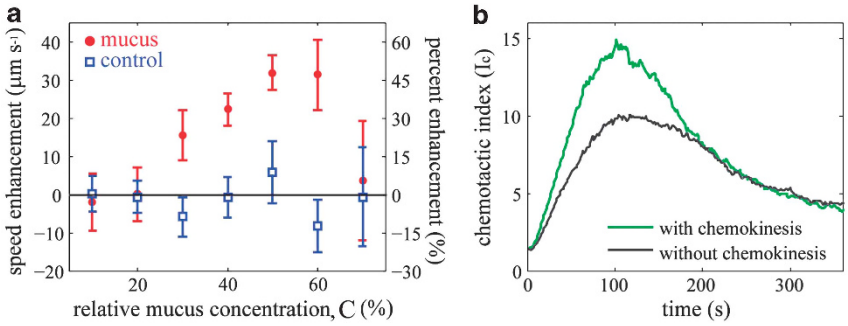

Figure 3 *V. coralliilyticus* exhibits chemokinesis that markedly increases the strength and speed of its response to coral mucus. (**a**) Swimming speed enhancement as a function of the mucus concentration instantaneously experienced by cells (Supplementary Figure S3). The enhancement is relative to the mean swimming speed in the absence of mucus (66 μm s^{-1}) and is expressed as both a speed difference (left axis) and a percent difference (right axis). Mucus concentrations, predicted from solution of the diffusion equation (Supplementary Information), are expressed relative to full mucus (i.e., the initial mucus concentration in the microchannel). Error bars represent the s.e. (**b**) A mathematical model of bacterial motility (Supplementary Information) shows that $I_{C,MAX}$ is 50% higher when chemokinesis in included (green) compared with the case of chemotaxis alone (gray), and that $I_{C,MAX}$ without chemokinesis is reached in <50% of the time when chemokinesis is present. Chemokinesis was modeled as a 24% enhancement of the mean swimming speed in regions with mucus concentrations greater than 20% of full mucus, based on the observed speed–concentration relationship (panel **a**).

expected owing to the natural variability in mucus composition among colonies. This variability notwithstanding, all responses observed with DMSP at comparable concentrations to those found in coral mucus were consistent with it being a primary driver of the chemotaxis. In addition, all responses observed were substantially faster and stronger than previously observed chemotactic responses of the enteric bacterium *E. coli* to its strongest chemoattractants and of the marine bacterium *P. haloplanktis* to algal exudates (Figure 2a). The same was true for *A. millepora* mucus when chemotactic responses to DMSP alone in mucus-equivalent concentrations were compared with responses to whole mucus (Supplementary Figure S2). These results demonstrate that DMSP within coral mucus is a major driver of the pathogen's behavior.

Chemotaxis by marine bacteria toward DMSP has previously been ascribed to DMSP's value as a rich carbon and sulfur source, as bacteria may obtain up to 15% of their carbon and most of their sulfur from DMSP (Zubkov *et al.*, 2001). Intriguingly, despite its vigorous swimming response toward DMSP, *V. coralliilyticus* does not detectably metabolize the compound. NMR analysis following a 6-day incubation of the pathogen in minimal media, where DMSP was either the sole carbon or the sole sulfur source, showed no degradation of DMSP by *V. coralliilyticus* (Supplementary Figure S7). The bacterium similarly did not degrade DMSP within whole-coral mucus (Figure 2b), confirming that it cannot cometabolize DMSP with other mucus-derived molecules. *V. coralliilyticus*' inability to metabolize DMSP, combined with its lack of genes homologous to any known DMSP degradation gene (Supplementary Table S2), indicates that DMSP is used by this pathogen purely as an infochemical, a function that DMSP and its derivatives also serve among pelagic reef fish (Debose and Nevitt, 2007) and marine protists (Seymour *et al.*, 2010; Garcés *et al.*, 2013). By demonstrating that the origin of the DMSP cue for *V. coralliilyticus* is its host, these results provide the first evidence that DMSP has a signaling role in the onset of a bacterial disease by aiding in the detection of a suitable host.

The importance of DMSP as a signaling cue, together with evidence that stressed corals are more susceptible to bacterial disease (Bruno *et al.*, 2007), prompted us to examine whether the mucus of stressed corals contains more DMSP and elicits stronger responses in *V. coralliilyticus* compared with unstressed controls. We performed a clonally replicated high-temperature stress experiment (temperature raised 1.5 °C/day over 6 days) on Heron Island. Analysis by quantitative NMR demonstrated a fivefold increase in DMSP concentration in the mucus of heat-stressed *P. damicornis* fragments (31 °C) compared with control fragments from the same colonies (maintained at 22 °C). No other molecule was substantially enriched in the stressed coral mucus compared with the controls, with the exception of DMSP's main degradation product, dimethylsulfide, toward which *V. coralliilyticus* chemotaxes only very weakly (Supplementary Figure S8). This result shows that DMSP may therefore be a strong cue for *V. coralliilyticus* to detect stressed or susceptible coral hosts. This latter hypothesis was supported by further chemotaxis experiments, which yielded a twofold higher I_C in response to mucus from stressed fragments ($I_{C,MAX} = 8$) relative to mucus from control fragments ($I_{C,MAX} = 4$) (Figure 2c; Supplementary Figure S6). The stress-induced enhancement of host-released cues and the simultaneous intensification of the pathogen's response strongly suggest that chemical signaling and active behavior represent important components in the pathway to disease. Furthermore, the widespread increase in susceptibility to

bacterial infection of stressed animals (Mydlarz *et al.*, 2006; Verbrugghe *et al.*, 2012) suggests that chemical interactions such as these may be a recurring element in other marine diseases.

The behavioral response of *V. coralliilyticus* to coral mucus and DMSP is not limited to chemotaxis, but includes a second, powerful behavioral adaptation: chemokinesis. Whereas chemotaxis is the ability to bias swimming direction in response to a chemical gradient, chemokinesis is the ability to change swimming speed in response to a change in chemical concentration. By analyzing trajectories of individual swimming cells (Supplementary Figure S3), we found that *V. coralliilyticus* exhibits a strong chemokinetic response to coral mucus (Supplementary Figures S4 and S9). *V. coralliilyticus* increases its mean swimming speed by 24% (from 66 $\mu m\,s^{-1}$ to 82 $\mu m\,s^{-1}$) in regions with $C > 20\%$ (where C is the local mucus concentration as a percentage of full mucus) and by up to 48% (98 $\mu m\,s^{-1}$) where $C \approx 60\%$ (Figure 3a). At even higher concentrations, cells slowed down (70 $\mu m\,s^{-1}$) possibly to retain their position near the source.

The rare ability of *V. coralliilyticus* to simultaneously employ chemotaxis and chemokinesis represents a powerful adaptation for responding to the chemical signals emanating from its coral host. Chemokinesis alone would result in the dispersion of cells away from regions of high chemical concentration because faster swimming cells have higher dispersal rates (Schnitzer, 1990). However, a mathematical model (Supplementary Information) reveals that, when paired with the ability to sense gradients, chemokinesis can substantially increase the chemotactic velocity and therefore reduce the time required to traverse a chemical gradient. A modest concentration-dependent increase in swimming speed (24%) nearly halved the timescale of the response to mucus (55 s vs 105 s to reach $I_C = 10$) and increased the peak response intensity by 50% ($I_{C,MAX}$ of 15 vs 10; Figure 3b; Supplementary Information). Although chemokinesis has been observed in a range of bacteria (Barbara and Mitchell, 2003; Seymour *et al.*, 2010), neither its disproportionate contribution to a bacterium's ability to climb chemical gradients nor its potential importance in an infection process have been previously reported. This observation indicates that chemokinesis is a powerful behavioral adaptation in *V. coralliilyticus*'s response to host-derived chemical signals and that bacterial infection of corals may be driven by considerably more specific adaptations than a binary presence or absence of motility (Meron *et al.*, 2009).

If DMSP represents a steady signal that seemingly provides ample time to locate the coral surface, why does *V. coralliilyticus* exhibit multiple, energy-intensive (Taylor and Stocker, 2012) motility adaptations of such magnitude? We hypothesize that the reason lies in the highly dynamic environment at the coral surface, where resident microbes contend for the best niches (Ritchie, 2006), mucus is periodically shed (Garren and Azam, 2012), surface cilia deter the attachment of fouling organisms (Wahl *et al.*, 1998) and external flows on the order of millimeters per second sweep over the colony (Lesser *et al.*, 1994). The residence time next to the surface is likely to be short, offering the pathogen only limited windows of opportunity for colonization of the host. In this complex environment, the ability to swim with high directionality (chemotaxis) and accelerate when accelerate when heightened concentrations are detected (chemokinesis) could aid *V. coralliilyticus* substantially in reaching the coral mucus.

Once a cell is within the mucus layer, it is only a short distance away from the coral tissue, and mucus itself is unlikely to slow its progress toward the tissue. This is supported by mathematical models (Spagnolie *et al.*, 2013) that predict that even when the medium is viscoelastic and has twice the viscosity of seawater, a bacterium with a single polar flagellum, such as *V. coralliilyticus*, will actually experience a minor increase ($< 5\%$) in swimming speed. The mucus layer is furthermore often very thin (a few hundred micrometers; Jatkar *et al.*, 2009), including for *P. damicornis* (Garren and Azam, 2012), requiring only seconds to traverse at typical swimming speeds. Finally, our experiments on chemokinesis, conducted with a 333 μm thick layer of whole mucus, showed no evidence

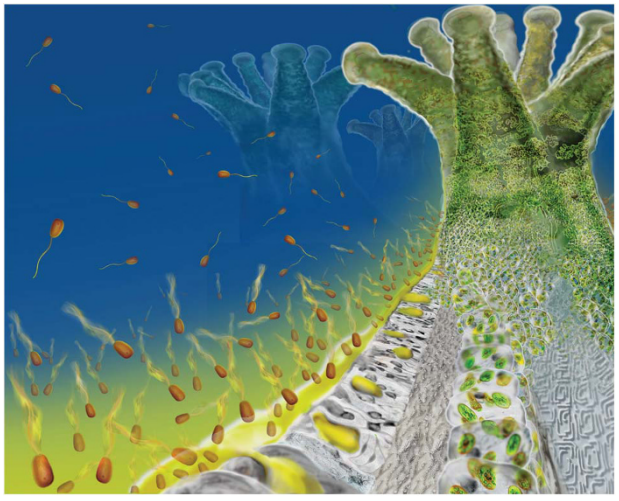

Figure 4 A new model for host detection by coral pathogens. The coral surface represents an intense source of molecules, such as DMSP, that diffuse (yellow gradient) away from the surface and out into the surrounding water, thereby establishing chemical gradients that motile bacterial pathogens (not to scale) can use to navigate toward their host. The striking prevalence of motility among putative coral pathogens (Supplementary Table S1), together with the strength of the chemical signals at the coral surface relative to typical signals in the water column, indicate that the advanced motility adaptations described here could be a widespread phenotype associated with coral disease.

for any decrease in motility. Rather these experiments demonstrated an up to 48% increase in speed at intermediate mucus concentrations. As a result, we expect that bacterial pathogens will have no physical difficulty penetrating the mucus layer.

Our findings reveal a previously unrecognized role of DMSP in the pathway to coral disease. The discovery that DMSP is involved in a pathogen's response to its host broadens the diversity of roles that this molecule has in the ocean (Kirst, 1989; Kirst *et al.*, 1991; Stefels, 2000; Sunda *et al.*, 2002; Debose and Nevitt, 2007; Simó *et al.*, 2009; Seymour *et al.*, 2010) to include that of a kairomone (a chemical that benefits the receiving but not the producing organism) and, together with the recent observation of a marine parasitoid using dimethylsulfide to locate its dinoflagellate host (Garcés *et al.*, 2013), suggests that sulfur compounds may be involved in host recognition in a broader class of marine infections. The surprising observation that *V. coralliilyticus* was unable to degrade DMSP, which is otherwise a rich carbon and sulfur source for bacteria (Zubkov *et al.*, 2001), suggests that the pathogen either has an unknown pathway for utilizing DMSP at exceedingly slow rates or it uses DMSP solely as a strong infochemical—our metabolic analyses point toward the latter explanation. The finding that hosts under heat stress exude mucus that is richer in DMSP and triggers heightened pathogen responses indicates that *V. coralliilyticus* may use this chemical cue to target stressed hosts. This emphasizes the risks posed to corals by warming waters and contributes to understanding the mechanisms underlying disease outbreaks associated with increasing seawater temperatures. Taken together, these observations unveil a strong role of microscale chemical ecology and microbial behavior in coral disease (Figure 4), emphasizing that the mechanistic drivers of many ecosystem processes may best be understood at the microscale level.

Conflict of Interest

The authors declare no conflict of interest.

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