1 **Coral mucus rapidly induces chemokinesis and genome-wide transcriptional shifts**
2 **toward early pathogenesis in a bacterial coral pathogen**
3

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22 **Competing Interests**
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Abstract

Elevated seawater temperatures have contributed to the rise of coral disease mediated by bacterial pathogens, such as the globally distributed *Vibrio coralliilyticus*, which utilizes coral mucus as a chemical cue to locate stressed corals. However, the physiological events in the pathogens that follow their entry into the coral host environment remain unknown. Here, we present simultaneous measurements of the behavioral and transcriptional responses of *V. coralliilyticus* BAA-450 incubated in coral mucus. Video microscopy revealed a strong and rapid 'chemokinetic' behavioral response by the pathogen, characterized by a two-fold increase in average swimming speed within six minutes of coral mucus exposure. RNA sequencing showed that this bacterial behavior was accompanied by an equally rapid differential expression of 53% of the genes in the *V. coralliilyticus* genome. Specifically, transcript abundance ten minutes after mucus exposure showed upregulation of genes involved in quorum sensing, biofilm formation, and nutrient metabolism, and downregulation of flagella synthesis and chemotaxis genes. After 60 minutes, we observed upregulation of genes associated with virulence, including zinc metalloproteases responsible for causing coral tissue damage and algal symbiont photoinactivation, and secretion systems that may export toxins. Together, our results suggest that *V. coralliilyticus* employs a suite of behavioral and transcriptional responses to rapidly shift into a distinct infection mode within minutes of exposure to the coral microenvironment.

Introduction

Coral reefs are declining worldwide due to rising sea surface temperatures and increasing prevalence of coral disease outbreaks (1–3). Elevated sea surface temperatures cause

47 physiological stress in corals (4) and provide distinct advantages for some coral pathogens
48 (5). One well-studied coral pathogen, *Vibrio coralliilyticus* BAA-450, displays a tightly
49 regulated temperature-dependent virulence against its coral host, *Pocillopora damicornis*.
50 While this *V. coralliilyticus* strain is avirulent at temperatures below 24 °C, it is capable of
51 attacking the coral symbiotic dinoflagellates (6,7) and lysing coral tissue (8) at temperatures
52 above 27 °C.
53
54 *V. coralliilyticus* displays two distinct behavioral adaptations enabling targeted infection of
55 corals that are physiologically stressed and therefore more vulnerable to pathogenic
56 invasion. First, the bacterial pathogen uses chemotaxis to target chemical signatures
57 present in the mucus of stressed corals (9). Second, *V. coralliilyticus* displays
58 chemokinesis, which is the ability to change swimming speed in response to a change in
59 chemical concentration to potentially enable faster environmental exploration in the
60 presence of its coral host mucus (9,10). While both chemotaxis and chemokinesis are
61 behaviors associated with motility and chemical sensing, chemotaxis specifically refers to
62 the ability to detect and follow chemical gradients (without necessarily any change in
63 swimming speed), whereas chemokinesis refers to the ability to change swimming speed in
64 response to an overall change in concentration in the environment (without any reference
65 to whether cells follow gradients). In *V. coralliilyticus*, these two behaviors combine to
66 enable efficient and rapid targeting of stressed corals. However, chemokinesis, in contrast
67 to chemotaxis, has remained more rarely studied in bacteria and is almost entirely
68 undescribed in the context of marine disease (11–13).
69

70 While coral mucus triggers increased motility and chemotaxis, which are behaviors
71 necessary for infection by *V. coralliilyticus* (14,15), the mucus also represents the critical
72 interface where pathogen activities can dictate the outcome of an infection. Corals secrete
73 up to half of the carbon assimilated by their algal symbionts as mucus (16,17) and its
74 production represents a sizable energetic investment that is important for nutrient cycling
75 across the entire reef system (18–21). In addition, mucus provides corals with protection
76 against desiccation and is an ancient and evolutionarily conserved first line of defense
77 against pathogens (22). During infection studies, corals have been observed to actively
78 expel ingested pathogens by spewing out bacteria-laden mucus from the polyps' mouths
79 (23,24). However, entry into host mucus may alternatively signal to the bacterial pathogen
80 that contact with a potential host is imminent. Thus, elucidating the behavioral and
81 transcriptional responses of *V. coralliilyticus* in the context of its coral host environment,
82 and in particular coral mucus, is important in elucidating the mechanisms underpinning
83 coral infection.
84
85 Here, we present experiments in which we simultaneously used video microscopy and RNA
86 sequencing to measure the behavioral and transcriptional responses of *V. coralliilyticus*
87 upon a sudden exposure to mucus from its coral host. To study chemokinesis
88 independently from chemotaxis, we conducted our experiments in the absence of chemical
89 gradients. This represents the first investigation to couple behavioral and transcriptomic
90 analyses to decipher the mechanisms promoting coral infection. We show that behavioral
91 and transcriptomic responses occur concomitantly over a surprisingly rapid timescale of
92 only minutes, highlighting the agility of the pathogens in seizing what are likely to be limited
93 windows of opportunity (25) to target and ultimately infect their host.

94 **Materials and methods**

95 **Coral mucus collection**

96 Five small colonies of the coral *Pocillopora damicornis* were collected from Heron Island
97 (23.4423°S, 151.9148°E, Great Barrier Reef, Australia) in April 2015 and maintained in
98 aquaria for two weeks at 25 °C ± 1 °C and 35 ppt salinity on a 12 h light–dark cycle. Mucus
99 was collected with a sterile 1-ml syringe (Becton Dickinson, NJ, USA) from corals subjected
100 to repeated 3-min air exposure, and snap-frozen in a sterile 50 ml tube (Falcon, Corning
101 Life Sciences, MA, USA) in liquid nitrogen and maintained at −80 °C until use in
102 experiments. Due to the large volume requirement (55 ml), mucus was collected and
103 pooled from the five coral colonies over three consecutive days (separate snap-frozen tube
104 per day). Directly before experimentation, a single pool of coral mucus was created by
105 thawing in a room temperature water bath and pooling samples in a sterile glass bottle.
106

107 **Bacterial culture**

108 *Vibrio coralliilyticus* type strain ATCC BAA-450 was acquired from the American Type
109 Culture Collection (Manassas, VA, USA). A frozen stock (−80 °C, 25% glycerol) of *V.*
110 *coralliilyticus* was streaked onto a marine broth 2216 (BD Difco, Franklin Lakes, NJ, USA)
111 culture plate and incubated at room temperature for 24–48 h. For liquid culture inoculation,
112 five colonies were resuspended in 20 µl filtered (0.2 µm) artificial sea water (FASW, 35 g/L;
113 Instant Ocean) and 5 µl of this suspension was inoculated into each of three sterile 250-ml
114 Erlenmeyer flasks containing 80 ml of 1% marine broth medium (99% FASW, v/v). The
115 timing of inoculation of the triplicate liquid cultures was staggered to allow 17–18 h of
116 growth (to OD_{600} 0.04) before the start of each replicate experiment. Liquid cultures were
117 incubated at 30 °C in an orbital shaker (250 rpm).

118

119 **Preparation of filtered spent medium**

120 Experimental controls consisted of *V. coralliilyticus* incubated in filtered spent medium,
121 which was prepared immediately prior (≤10 min) to the start of each replicate experiment.
122 Approximately 20 ml *V. coralliilyticus* culture was aseptically filtered through a sterile 0.2-
123 µm syringe filter (polyethersulfone membrane; VWR International) attached to a sterile 10-
124 ml syringe, into a sterile 50-ml Falcon tube. Filters were changed every 10 ml of volume
125 filtered to minimize clogging.

126

127 **Experimental setup**

128 Three biological replicate experiments were sequentially performed on the same day using
129 three independent cultures of *V. coralliilyticus* and their respective filtered spent media
130 (control) or pooled coral mucus (treatment) (Fig. 1). Aliquots of 25 ml of bacteria were
131 placed into each of two RNase-free 50-ml Falcon tubes for the mucus and control
132 treatments. Bacteria (before and after addition of mucus), coral mucus, and filtered spent
133 media were maintained at 30 °C ± 1 °C in a water bath for the duration of the experiment to
134 prevent temperature shock. All experiments were performed at 30 °C to capture conditions
135 in which *V. coralliilyticus* is capable of infecting its coral host (8). Incubation was initiated by
136 the 1:1 (v/v at t = 0 min) addition of coral mucus or filtered spent medium to bacteria,
137 directly followed by thorough mixing by repeated pipetting to eliminate any chemical
138 gradients.

139

140 Bacteria were repeatedly and manually sampled from populations incubated with mucus or
141 filtered spent media. At each time point, 40 µl of the bacteria-containing solution was

142 removed from the top of the incubation tube using a pipette and introduced into a straight
143 microfluidic channel (60 mm × 4 mm, 90 µm depth), followed immediately by video
144 acquisition. A single microfluidic device, fabricated using soft lithography (26) and
145 permanently bonded to a glass microscope slide (75 mm × 25 mm × 1 mm; VWR
146 International), contained two identical channels in parallel to accommodate the mucus and
147 the control experimental conditions. A temperature-controlled microscope stage insert
148 (Warner Instruments, Hamden, CT, USA) was used to maintain cells at 30 ± 1 °C during
149 imaging. After each time point, cells were removed from the device and discarded using a
150 pipet, before the introduction of freshly sampled cells at the next time point. A new
151 microfluidic device was used for each replicate experiment. Three biological replicate
152 experiments were performed sequentially on the same day, using three different cultures of
153 *V. coralliilyticus* exposed to aliquots of the same pooled coral mucus (Fig. 1).
154

155 **Microscopy and video analysis**

156 Experiments were imaged using phase contrast microscopy on an inverted epifluorescence
157 TE2000 microscope (Nikon, Tokyo, Japan), controlled through Nikon Elements software,
158 with a 20× objective (S Plan Fluor ELWD ADM Ph1 20×, 0.45 NA; Nikon) and 1.5× optical
159 magnification (combined objective magnification of 30×). An sCMOS camera (Andor Neo,
160 2560 × 2160 pixels, 6.5 µm/pixel; Andor, Belfast, Northern Ireland) was used to acquire
161 videos (300 frames at 29.79 fps) from the center of the microfluidic channels, with the focal
162 plane at channel mid-depth to avoid wall effects on motility.
163

164 Analysis of microscopy videos was performed in MATLAB (MathWorks, Natick, MA, USA)
165 using an automated image segmentation and trajectory reconstruction software developed

166 in-house (detailed methods in Supplementary Methods). Briefly, cells in each image were
167 identified using a pixel intensity threshold, and their swimming trajectories were
168 reconstructed using their *x,y*-positions in sequential frames (Fig. 2a,b). To discriminate
169 motile from non-motile bacteria, we determined the type of motion (ballistic or diffusive) of
170 each bacterium by calculating the mean squared displacement (MSD) as a function of short
171 time intervals (Δt), and quantifying the exponent α of this dependence (MSD ~ Δt^{α}). Non-
172 motile bacteria were identified as slow-moving cells (median instantaneous speed < 10
173 μm/s) displaying purely diffusive motion ($\alpha < 1$). Motile bacteria were instead identified as
174 those cells moving more rapidly (median instantaneous speed ≥ 10 μm/s) and having a
175 higher value of the MSD exponent ($\alpha \geq 1$) (Supplementary Fig. 1). A sensitivity analysis
176 showed that our results were robust against the selection of different threshold values
177 (Supplementary Fig. 2). Amongst motile bacteria, the swimming speed of each cell was
178 calculated by averaging the instantaneous speed over the duration of its trajectory, and the
179 mean speed of the population was quantified by averaging over all trajectories of motile
180 bacteria detected in each microscopy video (mean ± s.d. of number of trajectories in
181 videos, n = 1298 ± 312 pre-addition, n = 753 ± 230 post-addition; Supplementary Fig. 3),
182 representing a single time point in mucus or control conditions (Fig. 2).
183

184 **RNA sampling, isolation, sequencing, and sequence alignment**

185 To characterize changes in gene expression accompanying shifts in swimming behavior,
186 RNA sequencing (RNA-seq) was performed on the same *V. coralliilyticus* populations from
187 which samples for tracking by video microscopy were obtained. Incubation tubes containing
188 bacteria were swirled vigorously to mix before taking samples of mucus and control cells at
189 three time points during each replicate experiment (Fig. 1). Sample volumes of 8 ml pre-

190 mucus or -filtered spent medium (control) addition, and 16 ml post-addition (10 min and 60
191 min; larger volume to account for biomass dilution) were each filtered through a 0.22 µm
192 Sterivex-GP filter unit (polyethersulfone membrane; Millipore) attached to a sterile 60-ml
193 syringe to capture bacteria onto the membrane. Subsequently, the filtrate end of the
194 Sterivex filter cartridge was briefly flamed and pinched to close, and 2 ml of RNAlater
195 (Thermo Fisher Scientific) was added with a pipette through the other end of the cartridge
196 to immerse the cell-containing filter membrane in the RNA-stabilizing solution. Sample-
197 containing filter cartridges were incubated at 4 °C for 24 h, then preserved at −80°C until
198 RNA extraction. A sample of coral mucus (~2 ml) was also preserved, and its RNA
199 extraction confirmed that little to no RNA was present in coral mucus.
200
201 Total RNA extraction was performed as described previously (27) (detailed description in
202 Supplementary Methods). Depletion of rRNA (using Ribo-Zero rRNA Removal Kit for
203 bacteria (Epicentre Biotechnologies)) and subsequent mRNA sequencing (using Illumina
204 HiSeq2500 sequencer), assembly, and alignment were performed by the Joint Genome
205 Institute (Los Alamos, NM, USA) (detailed description in Supplementary Methods). Due to
206 RNA degradation, the mucus-treatment sample at 10 min from replicate 2 could not be
207 sequenced. Thus, 17 samples in total were sequenced (Sequence Read Archive accession
208 PRJNA707316). Raw reads from each library were aligned to the reference genome (*V.*
209 *coralliilyticus* ATCC BAA-450, NCBI Taxon ID 675814). As a result, 99.48% (5022 genes)
210 of the filtered FASTQ reads mapped to the *V. coralliilyticus* reference genome. GenBank
211 protein IDs (prefix “EEX”) were obtained from *V. coralliilyticus* BAA-450 genome assembly
212 ASM17613v1 and matched by locus tags (prefix “VIC_”) of each gene.
213

214 **Differential expression analysis**

215 The DESeq2 package (28) (v1.26.0) in R was used for differential expression analyses.
216 The DESeq2 algorithm uses negative binomial generalized linear models to test for
217 differential abundances in raw count data, and controls for differences in sequencing depth
218 between libraries by estimating size and dispersion factors. Adjusted *p*-values were
219 calculated in DESeq2 using the Benjamini-Hochberg procedure, which accounts for
220 multiple comparisons. Statistically significant gene expression differences were assessed
221 using the Wald test with a false discovery rate (FDR) adjusted p-value < 0.01. No fold
222 change cutoff was applied. Time-point-matched, pairwise mucus-control comparisons were
223 performed. All instances of gene differential expression given in the text are statistically
224 significant (unless otherwise noted), and the fold difference values provided in each case
225 are relative to the control at the same time point.

226

227 For principal component analysis (PCA) and sample-to-sample distance calculation, raw
228 count data were transformed using the variance stabilizing transformation (VST) method
229 (29) to remove the dependence of the variance on the count mean, especially when count
230 means are low. VST uses the experiment-wide trend of variance over mean in order to
231 transform the data to remove the experiment-wide trend. Transformed values are on the
232 $\log_2$ scale.

233

234 **Gene Set Enrichment Analysis (GSEA)**

235 Gene set enrichment analysis was performed using the GSEA software (30,31) v4.0.3 to
236 identify differential representation of metabolic pathways in transcripts sequenced in mucus
237 or control conditions. Only genes with significant differential expression determined by

238 DESeq2 were included in the GSEA analyses (GSEAPreranked protocol). Gene sets were
239 defined by KEGG Pathways, and significance was determined at an FDR *q*-value cut-off of
240 0.25. Genes were ranked by log_2 fold change values. Further details are described in
241 Supplementary Methods.
242

243 **Results**

244 ***V. coralliilyticus* exhibits strong chemokinesis within minutes of exposure to coral**
245 **mucus**

246 Video microscopy revealed a strong and rapid behavioral response by *V. coralliilyticus* cells
247 following exposure to *Pocillopora damicornis* coral mucus (Fig. 2) at 30 °C, a temperature
248 at which this pathogen is capable of infecting its coral host (8). The viscosity of coral mucus
249 in our experiments (0.750 cP) was similar to that of filtered artificial seawater (0.731 cP) at
250 room temperature (Supplementary Methods). Thus, bacterial responses measured in this
251 study were assumed to be mostly due to chemical components of coral mucus, although
252 temperature-dependent viscosity changes of coral mucus may influence bacterial
253 swimming (32,33).
254

255 *V. coralliilyticus* responded to coral mucus with strong chemokinesis. Video microscopy
256 revealed that the average swimming speed of *V. coralliilyticus* cells increased from 48.0 ±
257 3.4 μm/s (mean ± s.d.), measured five minutes before addition of coral mucus, to 81.0 ± 9.6
258 μm/s within two minutes of coral mucus addition (the first time point at which speed was
259 measured post mucus addition) (Fig. 2e). The maximum swimming speed (95.5 ± 4.6 μm/s)
260 was reached at six minutes post-addition, representing a two-fold increase compared to the

261 pre-addition state (Fig. 2e). After reaching their maximum speed, the average swimming
262 speed of bacteria in coral mucus gradually decreased over time, but remained significantly
263 higher than the swimming speeds of pre-addition and control cells for the entire
264 experimental duration (65 min) (two-tailed *t*-tests, $p < 0.01$). When samples were collected
265 for RNA-seq, at 10- and 60-min post-addition of mucus, swimming speeds were 90.1 ± 2.9
266 μm/s and 64.5 ± 0.9 μm/s, or 1.9× or 1.3× compared to baseline, respectively (Fig. 2e).
267
268 Chemokinesis by *V. coralliilyticus* is a temperature-dependent response and appears to be
269 driven by the influx of nutrients and other signaling molecules. First, coral mucus–induced
270 chemokinesis was attenuated in an experiment conducted at a lower temperature
271 (18.7 °C), in which the observed speed enhancement was only 1.1-fold at 7 min post-
272 mucus-addition (Supplementary Fig. 4). Second, in a separate experiment conducted at
273 30 °C, rich medium and coral mucus both led to a doubling of swimming speed (2.3-fold
274 increase for rich medium, 2.2-fold increase for coral mucus) within 5 min (Supplementary
275 Fig. 5), suggesting that, at least initially, chemokinesis is a response to the nutrient influx
276 upon addition of mucus. Third, control cells, exposed to filtered spent medium in place of
277 coral mucus, displayed a weak and short-lived increase in swimming speed that peaked at
278 1.3-fold the pre-addition speed after 8 min and returned to baseline within 15 min (Fig. 2e),
279 which may have been caused by oxygenation of the spent medium during filtration
280 (Supplementary Discussion). Taken together, our results suggest that the swimming speed
281 of *V. coralliilyticus* is highly sensitive to chemical changes in the environment, and that at
282 virulence-inducing temperatures (around 30 °C) chemokinesis in response to coral mucus
283 is especially rapid, large in magnitude, and long in duration, compared to any other
284 conditions tested.

Probability distributions of the swimming speeds of motile cells before and after mucus addition showed that the observed increase in average swimming speed was caused by a shift of the entire motile *V. coralliilyticus* population toward a faster swimming regime (Fig. 2c,d, Supplementary Fig. 6), rather than the emergence of behaviorally distinct subpopulations. In contrast, only a subset of motile control cells increased swimming speeds upon the addition of filtered spent media (Supplementary Fig. 6). Furthermore, approximately 75% of the population was motile over the whole experimental duration in both treatments (exposed to mucus or spent medium) (Supplementary Fig. 7). These results suggest that speed enhancement within the motile fraction of the population underlies the increase in average swimming speed observed in coral mucus, and that non-motile cells mostly maintain their non-swimming state upon mucus addition.

Within minutes of coral mucus exposure, *V. coralliilyticus* initiates a transcriptional response

Sequencing and alignment of mRNA libraries to the *V. coralliilyticus* BAA-450 reference genome, which contains 5078 protein-coding sequences (34), resulted in 5020 genes with non-zero total read count (Supplementary Tables 1, 2). Coral mucus led to a genome-wide response, with significant upregulation of 1379 genes (27% of detected genes) after 10 min, and 1159 genes (23%) after 60 min, relative to controls at the same time points (Fig. 3a). In addition, coral mucus exposure was associated with significant downregulation of 1326 genes (26%) after 10 min and 1076 genes (21%) after 60 min (Fig. 3a). Of all significantly differentially expressed genes, 1521 genes (30%) were shared between 10

307 and 60 min (Supplementary Table 3). These results suggest that exposure to coral mucus
308 leads to large shifts in the gene expression profile of *V. coralliilyticus*.
309
310 The changes in *V. coralliilyticus* gene expression following exposure to coral mucus
311 occurred rapidly and on the same time scales as the chemokinetic responses. Cells
312 exposed to mucus (10 min) displayed the largest transcriptomic shift in the principal
313 component analysis (PCA) space whose first two principal components captured 88.3% of
314 the variance (Fig. 3b). Replicate libraries clustered tightly together in the PCA space,
315 indicating little inter-replicate variance (Fig. 3b). Control and mucus cells at 10 min shared
316 similar transcriptional shifts along the second component (PC2, 15.9%, Fig. 3b), suggesting
317 that the transcriptional changes in control cells were also present in mucus cells. After 60
318 min of mucus incubation, gene expression occupied a distinct space on PC2 in comparison
319 with other time points, indicating a potential physiological switch that requires the
320 expression of a different group of genes than the early time point (10 min). Taken together,
321 our results reveal a rapid (within 10 min) transcriptional response of the pathogen to coral
322 mucus that mirrors the timescales of their behavioral changes.
323
324 Given the strong increase in swimming speed observed by video microscopy within the first
325 10 min of mucus exposure (Fig. 2), we searched for potential mechanisms underpinning
326 the chemokinesis behavior in the transcriptome. Our results from differential expression
327 analysis (DESeq2) revealed the upregulation of all six genes of the Na^+-translocating
328 NADH:ubiquinone oxidoreductase (Na^+-NQR) enzyme in coral mucus compared to controls
329 at 10 min (Fig. 4a, Supplementary Table 4). The Na^+-NQR enzyme participates in the
330 respiratory electron transport chain and is responsible for generating a sodium motive force

331 that drives flagellar rotation in Vibrios (35,36). Although the rapid onset (within 2 min) of
332 chemokinesis suggests that this behavior is not entirely a result of changes to gene
333 expression (bacterial protein production typically takes 10 minutes or longer (37)), the
334 increase in Na^+-NQR production may have enabled the sustained chemokinesis observed
335 over the duration of our experiments.
336
337 Surprisingly, chemotaxis genes and flagella genes were downregulated in coral mucus
338 compared to controls at 10 min (Fig. 3c, Supplementary Fig. 8), despite the strong increase
339 in swimming speed observed by video microscopy at the same time point (Fig. 2). Gene
340 Set Enrichment Analysis (GSEA) revealed that chemotaxis (KEGG 02030) and flagellar
341 assembly (KEGG 02040) gene sets were downregulated within 10 min of incubation with
342 coral mucus (Fig. 3c, Supplementary Fig. 8). All 17 chemotaxis *che* genes
343 (*cheABDRVWYZ*) were downregulated, except *cheX* (EEX30576) (Supplementary Fig. 8).
344 The majority of the 50 methyl-accepting chemotaxis protein (MCP) genes encoded in the *V.*
345 *coralliilyticus* genome were differentially expressed, with 26 downregulated and only 15
346 upregulated (Supplementary Fig. 8). None of the 74 flagellar assembly genes were
347 upregulated in coral mucus at 10 min, with all 31 differentially expressed genes
348 downregulated (Supplementary Fig. 8). Taken together, these patterns of gene expression
349 led us to speculate that coral mucus, while inducing a rapid and strong response of
350 chemokinesis, may also provide a cue for the transition from a motile to a non-motile (e.g.,
351 surface-associated) lifestyle.
352
353 *V. coralliilyticus* can grow on coral mucus, which, similar to other animal mucus, is rich in
354 sugars, lipids, peptides, amino acids (especially serine and threonine), and sulfur

355 compounds (38–42) (Supplementary Fig. 9). Metabolic pathways involved in the catabolism
356 of natural constituents of mucus were enriched in transcripts in mucus-treated cells at 10
357 min (Fig. 3c). Enriched pathways included metabolism of starch and sucrose (KEGG
358 00500), glycerophospholipid (KEGG 00564), glycine, serine and threonine (KEGG 00260),
359 sulfur compounds (KEGG 00920), and fatty acids (KEGG 00071) (Fig. 3c, Supplementary
360 Discussion). On the other hand, *V. coralliilyticus* transcriptomes after mucus treatment (10
361 mins) were depleted in functions involved in the assimilation of inorganic nitrogen (nitrate
362 and nitrite reductases in KEGG 00910; Fig. 3c), which may suggest a metabolic switch
363 toward the utilization of mucus-derived organic nitrogen (e.g. amino acids and peptides,
364 (39)). Almost all ribosomal protein genes (KEGG 03010) and aminoacyl-tRNA synthetases
365 (KEGG 00970) were upregulated in coral mucus compared to controls at 10 min (Fig. 3c,
366 Supplementary Fig. 10), which is indicative of elevated metabolism. Similarly, markers of
367 cell growth, *ftsZ* and *rpoD* (EEX34708 and EEX34866; Supplementary Fig. 11) were
368 upregulated by *V. coralliilyticus* cells exposed to coral mucus. This suite of changes
369 suggests that upon exposure to the nutrient-rich coral mucus, *V. coralliilyticus* rapidly
370 (within 10 min) and substantially alters its transcriptome to increase metabolism, protein
371 production, and growth.
372
373 Upregulation of functions associated with quorum sensing (KEGG 02024) and biofilm
374 formation (KEGG 05111) was observed in *V. coralliilyticus* cells exposed to coral mucus.
375 Upregulated genes included those involved in quorum-sensing signal molecule production
376 (*luxM*, *luxS*, *cqsA*) and regulation (*aphA*, *vtpR*), as well as biofilm-related polysaccharide
377 production (*vps* and *rbm* genes) (Fig. 4b–d). The *V. coralliilyticus* genes responsible for the
378 production of different quorum-sensing autoinducer molecules (AI-1, AI-2, CAI-1) (34)

379 displayed diverse but significant responses, with *luxM* (AI-1; EEX31502) upregulated and
380 *cqsA* (CAI-1; EEX33462) downregulated in mucus at both 10 and 60 min relative to
381 controls at the same time points (Fig. 4c). In contrast, *luxS* (AI-2; EEX35562) was first
382 downregulated at 10 min (0.35×) and subsequently upregulated at 60 min (1.8×) in mucus
383 relative to controls (Fig. 4c). Furthermore, the quorum-sensing master transcription
384 regulators, *aphA* (EEX30687) and *vcpR* (EEX34823; homologous to *luxR* of *V. harveyi* and
385 *hapR* of *V. cholerae* (43)), were up- and downregulated, respectively, which is consistent
386 with their reciprocal behavior in which AphA represses *vcpR* expression(44,45) (Fig. 4d). At
387 10 min in coral mucus, *aphA* was upregulated (2.8×) while *vcpR* was downregulated (0.5×)
388 compared to controls (Fig. 4d). In Vibrios, quorum sensing is tightly coupled with biofilm
389 formation (44,46–48). Indeed, the majority of the 18 *vps* (Vibrio polysaccharide) and 5 *rbm*
390 (rugosity and biofilm structure modulator) genes, which are essential for biofilm formation in
391 *V. cholerae* (49–52), were upregulated in coral mucus at 10 min (Fig. 4b). These early-
392 onset expression changes of specific genes involved in quorum sensing and biofilm
393 formation were followed by the significant upregulation of their entire pathways (39 quorum
394 sensing genes in KEGG 02024; 37 biofilm formation genes in KEGG 05111) at 60 min (Fig.
395 3d). Taken together, these results suggest that upon exposure to coral mucus, *V.
396 coralliilyticus* initiates a gene expression program for biofilm formation that may be involved
397 in host colonization.
398
399 Virulence genes characteristic of *Vibrio* pathogens were amongst the most strongly and
400 significantly upregulated genes in coral mucus (Supplementary Figs. 12, 13). The master
401 regulator of virulence, ToxR (EEX35320), and its associated stabilizer (53,54), ToxS
402 (EEX35319), were upregulated in coral mucus compared to controls at both the 10- and 60-

403 min time points (Fig. 4d). Concurrently, several toxin genes were upregulated in coral
404 mucus compared to controls at both time points (Fig. 4e). The important *V. coralliilyticus*
405 virulence factor, VcpB zinc metalloprotease (EEX32371), was one of the most strongly and
406 significantly upregulated genes in coral mucus at both 10 min (8.6×; Supplementary Figure
407 12) and 60 min (53.5×; Supplementary Figure 13). In addition, the VchA hemolysin
408 (EEX31069) and the associated putative chaperone VchB (EEX31068), which are
409 homologs of the primary virulence factors for *Vibrio vulnificus* (55,56), were significantly
410 upregulated in coral mucus at 60 min (3.9× and 11.8×, respectively) (Fig. 4e,
411 Supplementary Fig. 13). Furthermore, the upregulation of other zinc metalloproteases
412 (Supplementary Fig. 14) suggests the existence of multiple, as yet uncharacterized, zinc
413 metalloproteases available to *V. coralliilyticus* (Supplementary Discussion). Together, these
414 virulence factors and toxins may be responsible for the tissue lysis of corals previously
415 observed during *V. coralliilyticus* BAA-450 infection at elevated temperatures (8).
416
417 The bacterial secretion system (KEGG 03070) and protein export (KEGG 03060) gene sets
418 were significantly upregulated in coral mucus at 60 min (Fig. 3d), suggesting elevated
419 secretion of proteins. In particular, types 2, 6 (T2SS, T6SS) and Sec secretion system
420 genes were collectively upregulated in coral mucus (Fig. 4f). Indeed, *vipB* (EEX32048)
421 which encodes an essential component of T6SS (57), and *sec* genes were amongst the
422 most significantly and highly upregulated genes in coral mucus at 60 min (Supplementary
423 Fig. 13, Supplementary Table 5). In addition, the upregulation of β-lactam resistance
424 (KEGG 01501; Fig. 3d), as well as several of the multidrug-resistance efflux pump (*vex*)
425 genes (Supplementary Fig. 15), may confer resistance against antibiotic compounds
426 produced by commensal bacteria within coral mucus (58–63). Taken together, these results

427 suggest that exposure to coral mucus induces *V. coralliilyticus* to upregulate toxin
428 production, secretion and antibiotic resistance genes, which may be important for host
429 damage and for defense and competition against the commensal microbiome during host
430 colonization.

431 **Discussion**

432 We have reported a rapid behavioral and transcriptional response of *V. coralliilyticus* to
433 coral mucus exposure, which led to a two-fold increase in swimming speed and significant
434 differential expression of 53% of the genes in the genome within 10 minutes. Our findings
435 identify coral mucus as a potential chemical signal that induces pathogens to prepare for
436 host colonization and infection. These responses are in line with the behavioral and
437 physiological versatility characteristic of marine copiotrophic bacteria, which are often
438 adapted to boom and bust lifestyles (64,65), yet the extent and the rapidity of the
439 responses observed here suggest that temporally precise orchestration of behavior and
440 gene expression is important for coral host colonization by *V. coralliilyticus*.
441
442 Chemokinesis in response to exposure to coral mucus is potentially a strategy for *V.*
443 *coralliilyticus* to seize a limited window of opportunity to reach the coral surface. By
444 increasing swimming speed, bacteria also enhance their chemotactic velocity, leading to a
445 decrease in the time required to follow a chemical gradient to its source. This was
446 previously shown for *V. coralliilyticus* using microfluidic gradient experiments (9,10) and
447 appears to be a more general feature of Vibrios, having also been observed in *V.*
448 *alginolyticus* chemotaxing toward amino acids (13). While swimming fast is expensive in
449 the typically dilute ocean environment (66), energy is no longer limiting once nutrient-rich

450 mucus is available. Instead, what is limiting is the window of time that bacteria can exploit
451 that mucus signal to reach the host. Not only can ambient water currents transport bacteria
452 past the coral surface, but intense vortical flows produced by the corals themselves through
453 cilia on their surfaces – moving at speeds much greater than bacterial swimming speeds –
454 can result in rapid alternation of transport toward and away from the coral surface (25). In
455 this hydrodynamic environment, the colonization of a host by a bacterial pathogen is a
456 challenging behavioral feat, where the opportunity to home in and attach to the coral
457 surface may only last minutes or even less. The rapid response we reported here is
458 consistent with this dynamic environment. In particular, the strong chemokinesis – where
459 bacteria doubled their speed – is consistent with the need to reduce the time required to
460 migrate to the coral surface once the detection of mucus indicates the presence of a coral.
461 Furthermore, we observed that chemokinesis in response to coral mucus was almost
462 entirely absent at a temperature at which *V. coralliilyticus* is avirulent (18.7 °C), which is
463 consistent with the temperature-dependence of chemokinesis observed in our previous
464 study (10). Thus, we propose that chemokinesis is a virulence trait that is important for
465 successful host colonization by bacterial pathogens in the dynamic host surface
466 environment,
467
468 Entry into coral mucus represents a dramatic change in nutrient exposure for *V.*
469 *coralliilyticus* compared to the oligotrophic reef waters. Accordingly, *V. coralliilyticus* rapidly
470 upregulated metabolic pathways of nutrients that are present in coral mucus, which may
471 fuel the energetically expensive chemokinesis trait, as well as protein production (ribosome
472 and tRNA biosynthesis) and cell growth (*ftsZ* and *rpoD*) genes that may enable rapid
473 proliferation and confer a competitive advantage to pathogens as they invade the coral host

474 microbiome (67,68). Chemokinesis upon homogeneous addition of nutrients has been
475 observed in other bacteria including *Rhodobacter sphaeroides* (69), *E. coli* (70) and
476 *Azospirillum brasilense* (71), and it has been speculated that this swimming speed
477 enhancement is mediated by increasing the proton motive force that is responsible for
478 flagellar rotation (12,71). In line with this, *V. coralliilyticus* exposed to coral mucus
479 increased the expression, on a similar time scale as the chemokinesis behavior, of genes
480 encoding the Na^+-NQR enzyme, suggesting that regulation of periplasmic sodium levels
481 may help control swimming speed. Thus, we hypothesize that the metabolism of mucus
482 substrate stimulates Na^+-NQR activity, which in turn enables sustained chemokinesis.
483 Additional experimental work is required to test this hypothesis.
484
485 Despite the 1.88-fold increase in swimming speed observed through video microscopy,
486 flagellar genes were downregulated at the early RNA-seq time point (10 min). The
487 swimming phenotype may thus persist using the existing polar flagellum, while
488 downregulation of flagellar genes may be a strategy to prevent further replenishment of the
489 flagellar apparatus during the transition to a non-motile phase, evidenced by the concurrent
490 upregulation of biofilm genes. This observation has a parallel in the removal and
491 downregulation of flagella observed in pathogens within the human mucosa, where it is
492 speculated to be a strategy to escape immunological detection by the host, since flagella
493 are strong inducers of pro-inflammatory signaling (72). While corals possess innate and
494 adaptive-like immunity (73), whether a similar dynamic occurs on the coral surface is
495 currently not known.
496

497 Following only 10 minutes of exposure to coral mucus, the master regulator of *Vibrio*
498 virulence, ToxR, and its associated protein ToxS, were upregulated. ToxR is known to be
499 essential for coral infection by *V. coralliilyticus* (74,75), and in other *Vibrio* pathogens the
500 ToxR regulatory system coordinates the transcription of colonization, motility, and virulence
501 genes in response to environmental conditions (76,77). These downstream effects of ToxR
502 were indeed observed in our RNA-seq results. The temporal modulation of quorum-sensing
503 autoinducer molecules (AI-1, AI-2, CAI-1) as seen in our RNA-seq data may be a strategy
504 to coordinate metabolic and lifestyle transitions at the population level, as has been
505 observed in *Vibrio harveyi* (78). One such lifestyle transition may be biofilm formation,
506 which is tightly regulated by quorum sensing in *Vibrio* pathogens (47,79). Indeed, we
507 observed the upregulation of biofilm-related *vps* and *rbm* gene clusters in coral mucus at 10
508 min. Furthermore, we observed the upregulation of important *Vibrio* toxins, VcpB zinc
509 metalloprotease, and VchA and VchB hemolysins, in coral mucus at both 10- and 60-min
510 time points. Similarly, several secretion systems were upregulated, including the Sec-
511 dependent and type 2 secretion systems, which are together responsible for extracellular
512 secretion of a broad range of proteins, including toxins and degradative enzymes involved
513 in the pathogenesis of many Gram-negative bacteria (80–82). Type 6 secretion systems
514 are responsible for the injection of toxic effector proteins into bacterial cells in antagonistic
515 interactions (83–85). Taken together, the upregulation of *toxR* and *toxS*, as well as their
516 downstream gene expression effects, suggest that coral mucus serves as an environmental
517 signal for *V. coralliilyticus* to activate host colonization and virulence gene expression
518 programs.
519

520 The VcpB zinc metalloprotease is a key virulence factor of *V. coralliilyticus* that causes
521 photoinactivation of coral endosymbionts and coral tissue lesions (7), and its rank as one of
522 the most strongly and significantly upregulated genes in our RNA-seq dataset suggests that
523 the bacterium rapidly responded to coral mucus as a cue to initiate its virulence program.
524 However, the second zinc metalloprotease that has been implicated in *V. coralliilyticus*
525 infections of corals, VcpA (EEX33179) (8), was downregulated in our experiment. The two
526 zinc metalloproteases (VcpA and VcpB) may thus play redundant roles in *V. coralliilyticus*
527 infections and may be important in different environmental contexts (Supplementary
528 Discussion).
529
530 Our results underscore the rapidity of behavioral and transcriptional changes that occur in a
531 coral pathogen upon entry into the host environment (Fig. 5). These changes in swimming
532 and gene expression patterns paint a clear sequence of events immediately preceding
533 infection – although further validation with direct phenotypic evidence is required. Upon
534 exposure to coral mucus, the coral pathogen *V. coralliilyticus* (known to chemotax towards
535 coral mucus (9)) increases swimming speed by up to two-fold within minutes, a response
536 that, in the natural environment, would lead to faster chemotaxis and a halving of the time
537 required for the pathogen to track the coral surface from which the mucus signal originates.
538 This capacity to rapidly chemotax into the coral surface microenvironment is important
539 because of the short window of opportunity that the pathogens have in the hydrodynamic
540 environment surrounding corals. Simultaneously, transcriptional changes indicate that
541 mucus exposure immediately prompts *V. coralliilyticus* to increase nutrient metabolism and
542 prepare for host colonization and damage. The downregulation of motility genes, puzzling
543 at first in view of the strong chemokinetic response, is in fact consistent with the

544 upregulation of quorum sensing and biofilm formation genes, together suggesting a 'final
545 dash' to the coral surface enabled by enhanced swimming speed, followed by a rapid
546 transition to a non-motile, coral surface-associated lifestyle. The upregulation of
547 metabolism, growth, and antibiotic resistance genes suggests that the pathogen takes
548 advantage of mucus as a conspicuous energy source and prepares to colonize the coral
549 surface and compete with commensal bacteria. The upregulation of host damage genes
550 and secretion systems responsible for toxin export suggests preparation for the infection
551 process itself. Precise temporal control of pathogenesis is a hallmark of *Vibrio* pathogens
552 (86,87), which are capable of rapidly modulating their lifestyle between free-swimming and
553 biofilm phases in response to their environment, in particular temperature changes
554 (34,86,88). The frequency of acute temperature-rise in reef waters is increasing (89), giving
555 additional opportunities for temperature-dependent bacterial pathogens, such as *V.*
556 *coralliilyticus*, to infect corals (90,91). In this context, understanding the mechanisms
557 underlying the earliest stages of bacterial infections is critical in anticipating future disease
558 outbreaks and curbing coral mortality to protect the ecosystems that they support.
559

560 **Data Availability**

561 The data that support the findings of this study are available from the corresponding
562 authors on request (total data size approximately 2 TB). Raw, filtered sequencing data
563 reported in this paper have been deposited in the Sequence Read Archive (accession
564 PRJNA707316).

565

566 **Code Availability**

567 All computer code (in MATLAB and R) developed for this study is available from the
568 corresponding authors on request.

569

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584

585 **Author contributions**

586 C.G., M.G., K.P., J.R.T., and R.S. designed the study. C.G. and K.P. performed
587 experiments. J.B.R. collected coral mucus. C.G., V.I.F., and R.S. created the video analysis
588 software. C.G. performed RNA-seq data analyses. J.R.S. and M.G. provided conceptual
589 guidance. C.G., J.B.R., and R.S. wrote the manuscript. All authors edited the manuscript
590 before submission.

591

592 **Competing Interests**

593 The authors declare no competing interests.

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Figure Legends

Figure 1 | Behavioral and transcriptional measurements of *V. coralliilyticus* in coral mucus. Experimental timeline showing time points for microscopy video acquisition and RNA-seq sampling. At $t = 0$ (red arrow), coral mucus or filtered spent medium (control) was added (1:1, v/v) to *V. coralliilyticus* cultured in 1% marine broth. The experiment was conducted at 30 °C, and repeated three times sequentially in a single day, using three different cultures of *V. coralliilyticus*.

Figure 2 | *V. coralliilyticus* exhibits strong chemokinesis upon exposure to coral mucus. Experiments were conducted at 30 °C. (**a**, **b**) Swimming tracks of *V. coralliilyticus* before (–3 min, **a**) and after (10 min, **b**) addition of coral mucus. Swimming tracks of 70 motile cells were randomly selected for each panel. Black circles mark the start, and colors indicate the mean swimming speed, of each track. Colored circles (3× zoom of gray box provided for visibility) represent frames of a microscopy video (0.03 seconds per frame). (**c**, **d**) Probability distributions of swimming speeds of motile cells, before (–3 min, **c**) and after (10 min, **d**) addition of coral mucus (orange) or filtered spent medium (gray). (**e**) Average swimming speeds of motile cells before and after 1:1 addition (v/v at $t = 0$ min) of coral mucus (orange) or filtered spent medium (black, control). Averages (filled circles) and standard deviation (shaded regions) were calculated using data obtained from three replicate experiments (○, *, □). Data were interpolated to match time points across replicates. RNA-seq samples were taken at −5, 10, and 60 min (green circles). Data from replicate 3 are presented in panels **a–d**.

860 **Figure 3 | Exposure to coral mucus leads to genome-wide transcriptional shifts in *V.***
861 ***coralliilyticus*.** (**a**) Number of genes that were significantly upregulated or downregulated
862 in coral mucus at each time point (FDR $\alpha < 0.01$). (**b**) Principal component analysis (PCA)
863 plot showing all RNA-seq samples in the 2D plane spanned by the first two principal
864 components. PCA was performed using raw read count data after variance stabilizing
865 transformation. (**c**, **d**) Gene set enrichment analyses (GSEA) on mucus vs. control at 10
866 min (**c**) and 60 min (**d**)**.** Gene sets (KEGG pathways, bar labels) that were significantly
867 upregulated or downregulated in coral mucus (FDR $q < 0.25$) are shown, and their bars are
868 ordered top to bottom by FDR *q*-values (smallest to largest; i.e., most significant to least
869 significant) within each expression category (upregulated in mucus, yellow/orange, or
870 downregulated in mucus, gray). Number of significantly differentially expressed genes in
871 mucus that were included in the GSEA (bold), and the total number of genes in the *V.*
872 *coralliilyticus* genome assigned to the KEGG pathway, are shown. Orange bars represent
873 gene sets that were upregulated in mucus at both 10 and 60 min.
874

875 **Figure 4 | Differential expression of Na^+-NQR enzyme, surface association, and host**
876 **damage genes in coral mucus.** Log_2-transformed fold difference between mucus and
877 control (log_2 FD) and their adjusted *p*-values were determined using DESeq2. (**a**) Na^+**-**NQR
878 genes *nqrA–F* were identified through homology with *nqr* genes of *Vibrio alginolyticus*
879 (Supplementary Table 6). (**b**) *Vibrio* biofilm genes in the *vps* and *rbm* gene clusters were
880 identified through homology with *V. cholerae* genes (Supplementary Table 7). *Vps* genes
881 (*vpsU, vpsA–P*, *vpsR*, *vpsT*) (**a**) and *nqr* genes (*nqrA–F*) (**b**) are labeled with their
882 respective suffix letters. Lengths of arrows are proportional to protein size (scale bar; aa =
883 amino acids). (**c–e**) Log_2 FD of quorum-sensing autoinducer synthase genes (**c**), *Vibrio*

884 master transcription regulators (**d**), and toxins (**e**) at 10 and 60 min. Homology was found
885 with genes in other Vibrios (Supplementary Table 8). Fold difference before addition was
886 assumed to be 1:1 (mucus:control, $\log_2$ FD = 0). Error bars represent standard error
887 estimates for the $\log_2$ FD values. (**c**) Heatmap showing differential expression of secretion
888 system genes identified through KEGG pathway assignments (Supplementary Table 9).
889 The *V. coralliilyticus* genome has two sets of type 3 secretion genes (34). CPI-1,
890 Coralliilyticus Pathogenicity Island-1. Colors (**a**,**b**,**f**) indicate $\log_2$ FD values. Asterisks
891 (**a**,**b**,**f**), black outlines of arrows (**a–b**), and closed circles (**c–e**) mark genes with significant
892 differential expression (adjusted $p < 0.05$). Open circles, not significant.
893
894 **Figure 5 | Putative infection timeline of *V. coralliilyticus*.** Our results suggest that
895 exposure to coral mucus triggers a suite of behavioral and transcriptomic responses in *V.*
896 *coralliilyticus* leading up to infection. Within two minutes, coral mucus induces strong
897 chemokinesis, which allows the pathogens a faster final dash toward the coral surface via
898 chemotaxis (9,10). Early upon coral mucus exposure, upregulation of genes for metabolism
899 of mucus components, biofilm formation, quorum sensing, and antibiotic resistance, and
900 downregulation of flagella- and chemotaxis-related genes, enable host colonization and
901 competition with commensal bacteria. Toxin genes (zinc metalloproteases and hemolysins;
902 yellow stars) and secretion system genes are upregulated in coral mucus, which may lead
903 to host tissue and symbiont damage. Solid arrows indicate bacterial responses for which
904 we have direct observational evidence; dotted arrows indicate hypothesized phenomena
905 based on our RNA-seq data. Figure adapted from Garren et al., 2014 (9).