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REPORT

Visualization of coral host–pathogen interactions using a stable GFP-labeled *Vibrio coralliilyticus* strain

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Abstract The bacterium *Vibrio coralliilyticus* has been implicated as the causative agent of coral tissue loss diseases (collectively known as white syndromes) at sites across the Indo-Pacific and represents an emerging model pathogen for understanding the mechanisms linking bacterial infection and coral disease. In this study, we used a mini-Tn7 transposon delivery system to chromosomally label a strain of *V. coralliilyticus* isolated from a white syndrome disease lesion with a green fluorescent protein gene (GFP). We then tested the utility of this modified strain as a research tool for studies of coral host–pathogen interactions. A suite of biochemical assays and experimental infection trials in a range of model organisms confirmed that insertion of the GFP gene did not interfere with the labeled strain's virulence. Using epifluorescence video microscopy, the GFP-labeled strain could be reliably distinguished from non-labeled bacteria present in the coral holobiont, and the pathogen's interactions with the coral host could be visualized in real time. This study demonstrates that chromosomal GFP labeling is a useful technique for visualization and tracking of coral pathogens and provides a novel tool to investigate the role of *V. coralliilyticus* in coral disease pathogenesis.

Keywords *Vibrio coralliilyticus* · Green fluorescent protein · Coral disease · Host–pathogen interactions · Pathogens · Bacteria

Communicated by Biology Editor Dr. Ruth Gates

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Introduction

Coral reefs provide critical goods and services to tropical nations worldwide, but increasing levels of coral disease

threaten to erode the very foundation of these valuable ecosystems (Bourne et al. 2009). In spite of increasing research efforts, relatively little is known about the complex interactions among the coral host, the marine environment, and invading pathogens that lead to disease (Work and Meteyer 2014). The development of tools to effectively visualize pathogens in situ has provided critical insights into complex and highly dynamic host–pathogen interactions in a wide range of disease systems (Valdivia et al. 1996; Ling et al. 2001; Sawabe et al. 2006) and could lead to similar advances in the field of coral disease research.

Insertion of green fluorescent protein (GFP) genes into the genomes of microorganisms of interest provides a useful experimental tool to track the behavior of specific microbes as they interact with host tissues and cells (Prasher et al. 1992; Chalfie et al. 1994; Valdivia et al. 1996; Ling et al. 2001; Dunn et al. 2006). GFPs are non-toxic; therefore, they do not interfere with cell function. Moreover, they are continuously synthesized within modified pathogens and are passed to offspring during binary fission (Chalfie et al. 1994; Valdivia et al. 1996). These characteristics make genomic GFP insertion an important tool for microbiologists studying environmental microorganisms in complex biological systems (Errampalli et al. 1999). Development of tools to label and track coral pathogens could provide similar insights into pathogen invasion pathways and could facilitate the discovery of methods to control coral diseases, such as the identification of probiotics that protect the coral holobiont from infection. While several coral pathogens have been identified to date, techniques to track their dynamic interactions with coral hosts in vivo are currently lacking (Pollock et al. 2011).

The bacterium *Vibrio coralliilyticus* has recently emerged as a model pathogen for investigations into the mechanisms linking bacterial infection and coral disease in experimental settings (Meron et al. 2009; Pollock et al. 2010; Kimes et al. 2011; Garren et al. 2014). Although a direct link between *V. coralliilyticus* and widespread coral disease outbreaks has not been definitively established, this bacterium has been implicated in coral tissue loss diseases, collectively known as white syndromes (WS), at sites across the Indo-Pacific (Ben-Haim et al. 2003; Sussman et al. 2008; Ushijima et al. 2014). Additionally, aquarium-based infection experiments have demonstrated the ability of *V. coralliilyticus* to cause WS-like disease signs in several Indo-Pacific coral species (Sussman et al. 2008; Vidal-Dupiol et al. 2011; Ushijima et al. 2014). This potentially pathogenic bacterium is easy to culture, and several strains have been isolated from diseased corals that could be modified to allow specific visualization and tracking (Ben-Haim and Rosenberg 2002; Ben-Haim et al.

2003; Thompson et al. 2005; Sussman et al. 2008; Vizcaino et al. 2010; Ushijima et al. 2014). Movement patterns of this bacterium have been accurately tracked within chemical gradients mimicking the microenvironment on a coral's surface using microfluidic devices (Garren et al. 2014). However, in situ tracking on a coral's surface, where complex microtopography and bacterial communities render tracking more difficult, requires the development of novel tools, such as specific labeling.

In this study, we describe the chromosomal GFP labeling of a strain of *V. coralliilyticus* (LMG 23696) that was originally isolated from a WS-affected specimen of the coral *Montipora aequituberculata* on the Great Barrier Reef (GBR), Australia. Using a suite of biochemical assays and infection experiments, we confirm that there is no loss of virulence in this modified strain, and we employ epifluorescence video microscopy to visualize its interactions with the coral host in situ. This study demonstrates the efficacy and utility of GFP-labeled pathogens to investigate host–pathogen interactions within the coral holobiont.

Materials and methods

GFP labeling of *Vibrio coralliilyticus*

Vibrio coralliilyticus strain P1 (LMG 23696) was originally isolated from a WS-affected colony of the scleractinian coral *M. aequituberculata* at Magnetic Island, which is located off the coast from Townsville, Australia, within the central section of the GBR Marine Park (Sussman et al. 2008). The target strain of *V. coralliilyticus* was GFP labeled using the mini-Tn7 system that integrates the GFP gene into a neutral site of the bacterial chromosome, as previously described by Lambertsen et al. (2004). Briefly, relevant bacterial strains were grown in LB20 broth (3 g L^{-1} peptone, 1 g L^{-1} yeast extract, 20 g L^{-1} NaCl), supplemented with the appropriate antibiotics (see below) and cultured at 30 °C for 24 h with shaking (170 rpm). The GFP delivery vector pAKN137 was grown in 15 μg mL^{-1} gentamycin; the transposase delivery plasmid pUXBF13 was grown in 50 μg mL^{-1} ampicillin, and the mobilization plasmid pRK600 was grown in 5 μg mL^{-1} chloramphenicol. *V. coralliilyticus* P1 was routinely grown in 50 μg mL^{-1} colistin. After 24 h, strains were subcultured into fresh LB20 broth supplemented with the appropriate antibiotics and were cultured for 16 h at 30 °C with shaking (170 rpm). Strains were subsequently mixed and spotted onto LB20 agar plates (10 g L^{-1} peptone, 5 g L^{-1} yeast extract, 20 g L^{-1} NaCl, and 15 g L^{-1} agar) with no antibiotics. Transconjugants were selected on LB20 plates containing 15 μg mL^{-1} chloramphenicol and 50 μg mL^{-1} colistin and confirmed under a blue light transilluminator.

Biochemical and phenotypic validation of GFP-tagged
Vibrio coralliilyticus

GFP-labeled and wild-type (WT) strains of *V. coralliilyticus*
were compared to assess the impact of GFP gene insertion on
bacterial growth dynamics, proteolytic activity, and protein
expression. Growth rates of duplicate cultures of each strain
were compared using the microgrowth assay developed by
Brewster (2003). The cell density of each strain was adjusted
to the same starting concentration, and cell density mea-
surements (optical density at 595 nm [OD_{595}]) were taken
every hour using a Wallac 1420 Victor 2 spectrophotometer
(PerkinElmer Life Sciences, USA).

Virulence of *V. coralliilyticus* results in part from high
protease expression levels (Ben-Haim et al. 2003; Sussman
et al. 2009). Therefore, proteolytic activity and protein
expression profiles of the GFP-labeled and WT strains were
compared to ensure that no attenuation had occurred as a
result of the labeling process. Protease activity was quan-
tified by measuring azocasein hydrolysis, as previously
described by Windle and Kelleher (1997). After overnight
growth in LB20 broth, 1 mL of culture was pelleted by
centrifugation at $16,000 \times g$ for 10 min, and the supernatant
was sterilized through a 0.22 μm filter to remove any
bacterial cells. Cell-free supernatant was mixed with azo-
casein (5 mg mL^{-1} in 50 mM Tris–HCl pH 8.0 supple-
mented with 0.04 % NaN$_3$) and incubated at 30 °C for
60 min. Following incubation, the non-hydrolyzed protein
was precipitated using 10 % tricholoroacetic acid (TCA)
and removed by centrifugation. The supernatant was
transferred to a new tube, and the absorbance at 450 nm
was measured.

Symbiodinium infection study

To assess the impact of GFP labeling on *V. coralliilyticus*
virulence against endosymbiotic algae typically associated
with the coral host, axenic cultures of *Symbiodinium* were
challenged with WT and GFP-labeled strains of *V. coral-
liilyticus*, following procedures described in Cohen et al.
(2013). Briefly, *Symbiodinium* cultures were maintained in
an axenic growth medium comprised of a modified *F*/2 and
Erdschreiber medium (Guillard and Ryther 1962). *Sym-
biodinium* cells were quantified ($n = 10$) using a Neubauer
hemocytometer, adjusted to a uniform concentration
(1×10^6 cells mL^{-1}) by medium addition and inoculated
into sterile 96-well plates (100 μL per well), and wells
were covered and sealed with Parafilm®. Cell culture plates
were then incubated at 28 °C under 12-h light/dark irra-
diance (120 pmol photons m^{-2} s^{-1}).

Overnight cultures of WT and GFP-labeled *V. coralli-
ilyticus* were centrifuged at $5250 \times g$ for 10 min, and the
resulting supernatants were individually filtered through

0.22 μm filters to remove any bacterial cells. For each
bacterial strain, 100 μL of cell-free supernatant was then
added to four replicate microtiter wells containing axenic
Symbiodinium cells. Additionally, 100 μL of sterile filtered
seawater was added to four replicate wells as a negative
control.

A maxi imaging pulse amplitude modulation (MAXI
iPAM) fluorometer was used to assess photosystem II in-
hibition. Briefly, *Symbiodinium* cells were dark adapted for
30 min prior to each saturation light pulse (gain = 1–2,
intensity = 1–2, saturation pulse = 7) to obtain dark-
adapted quantum yields (F_v/F_m), which were calculat-
ed using the formula, $F_v/F_m = (F_m - F_0)/F_m$ with
F_v = variable fluorescence, F_m = maximum fluorescent
yield, and F_0 = dark fluorescent yield. Measurements were
collected prior to inoculation (i.e., time = 0), approximately
hourly for the first 5 h post inoculation and then
approximately every 2 h up to 26 h post inoculation.

Acropora millepora coral juvenile infection study

To assess the impact of GFP labeling on *V. coralliilyticus*
virulence against the coral holobiont, juveniles of the coral
Acropora millepora were challenged with both WT and
GFP-labeled strains of *V. coralliilyticus*, as previously
described by Cohen et al. (2013). *Acropora millepora* ju-
veniles were raised from larvae, as described by Abrego
et al. (2008) and Littman et al. (2010). Briefly, colonies of
A. millepora were collected from Cattle Bay, Orpheus Is-
land, in the central sector of the GBR prior to spawning in
November 2010. Following spawning and fertilization,
larvae were reared at Orpheus Island Research Station until
settlement competency was attained. After settlement onto
field-conditioned terracotta tiles, coral juveniles were re-
turned to a nearby reef on the west side of Pelorus Island.
Terracotta tiles with attached coral juveniles were placed
on steel rods and suspended vertically between pairs of
metal star pickets on the reef flat. After 6 months, the
terracotta tile racks were removed from the reef and
transported to the Australian Institute of Marine Science
(AIMS) in large seawater filled containers. At AIMS, the
terracotta tiles (and associated coral juveniles) were placed
in outdoor aquaria facilities with 5 μm filtered flow
through seawater for 1 week to allow for acclimatization.
After 1 week, juveniles were removed from the tiles using
a microscope, scalpel, and tweezers and placed into indi-
vidual wells in a 12-well plate. Each well contained 5 mL
of 0.22 μm filter-sterilized seawater, which was replaced
every other day. All plates containing juveniles were in-
cubated at 24 °C under 12-h light/dark photoperiods, with
irradiance of 120 pmol m^{-2} s^{-1}. Juvenile health assess-
ments were performed every two days to evaluate pig-
mentation and general health state. Juveniles displaying

signs of stress (i.e., bleaching or tissue loss; <10 % of juveniles) were removed from the experiment. Five days after collection, seawater temperature was slowly elevated by 2 °C–26 °C, and five days later, the temperature was further increased gradually to 28 °C. *Vibrio coralliilyticus* is a temperature-dependent pathogen shown to elicit maximal coral damage at temperatures exceeding 26 °C (Ben-Haim et al. 2003). Water temperature was elevated to enhance *V. coralliilyticus* virulence.

Overnight cultures of WT and GFP-labeled *V. coralliilyticus* ($OD_{595} = 0.8$) were centrifuged at $5250 \times g$ for 10 min, and the resulting supernatants were individually filtered through 0.22-μm syringe filters. Resulting cell-free supernatants were diluted 1:1 in 0.22 μm filter-sterilized seawater, and 5 mL of the resulting supernatant solution was used to replace the filtered seawater in three replicate wells, each containing one juvenile *A. millepora*, per strain. Additionally, 5 mL of 0.22 μm filter-sterilized seawater was added to three replicate wells each containing one coral juvenile as a negative control. Dark-adapted quantum yield measurements (as described above) were collected at time 0, approximately hourly for the first 3 h post inoculation and then approximately every 3 h up to 9 h post inoculation.

Aiptasia sp. infection study

To assess the impact of GFP labeling on *V. coralliilyticus* virulence against the sea anemone *Aiptasia* sp., a model organism for studies of coral genetics and physiology (Weis et al. 2008), anemones harvested from the AIMS seawater system were challenged with both WT and GFP-labeled strains of *V. coralliilyticus*. Anemones growing in the flow through aquarium system at AIMS were gently separated from the aquarium wall with the dull edge of a razor blade and placed into nine aquaria (each 6 L in volume) under 12-h light/dark irradiance (120 pmol photons m^{-2} s^{-1}) and at 28 °C, with three anemones per aquaria. Fifty percent water exchanges were performed every other day. Following a 2-day acclimation period, 1 mL of WT or GFP-labeled overnight cultures of *V. coralliilyticus* (pelleted, washed twice, and resuspended with sterile 0.22 μm filter-sterilized seawater) or filter-sterilized seawater (negative control) was injected directly into the tissue of nine individual anemones in three replicate tanks (three anemones per tank) for each treatment. Dark-adapted quantum yield measurements (as described above) were collected at time 0 and every other day for six days.

Host–pathogen visualization

Epifluorescence video microscopy was employed to assess the utility of the GFP-labeled strain for visualizing host–pathogen interactions in situ. Visualization trials were conducted using the GFP-labeled *V. coralliilyticus* P1 strain and WT *V. coralliilyticus* strain BAA-450 acquired from the American Type Culture Collection (www.atcc.org, Manassas, Virginia, USA) as a non-fluorescent control. Cultures were inoculated into 2216 Marine Broth, grown for 18 h at 30 °C while shaking at 300 rpm. Small colonies of the coral *Pocillopora damicornis* were cultured at 25 °C in artificial seawater (Instant Ocean, Spectrum Brands Company, Cincinnati, OH) on a 12-h light/dark cycle. Small branches of *P. damicornis* (<10 mm length; <5 mm diameter) were clipped from the parent colony, allowed at least 48 h to recover in the tank and subsequently used for microscopy.

Coral fragments were placed in individual chambers of a 4-well coverslip bottom chamber slide (LabTek^{TM}, Thermo Fisher Scientific, Waltham, MA, USA) with 1 ml of unfiltered aquarium seawater. Images were acquired using both phase contrast and epifluorescence video microscopy with a 20 × objective on a Nikon Ti microscope (Nikon, Tokyo, Japan) equipped with an Andor Neo or iXon CCD camera (Andor, Belfast, Northern Ireland) and the Nikon Elements software platform. Fragments were imaged (i) alone with the natural microbial assemblage growing on their surface and in the seawater, and subsequently, (ii) with the addition of 250 μL of bacterial culture (either the non-fluorescent control strain or the GFP strain at 10^6 cells mL^{-1}). Time-lapse projections of these videos were used to visualize the tracks of bacteria and other particulates in the fluid. The spatial distribution of individual bacteria in association with the host's surface was obtained by imaging a given field of view at multiple depths (z-stacks), with a 2 μm z-distance among consecutive images using the Nikon Elements software platform.

Statistical analyses

Differences in growth curves and photosystem II quantum yield (*Symbiodinium*, coral juvenile, and *Aiptasia* sp. infections) were assessed using repeated measures ANOVAs, and differences in protease activity were assessed using a one-factor (*Vibrio* strain/treatment) ANOVA. All post hoc comparisons were made using Tukey's honestly significant difference (HSD) analyses. Statistical analyses were performed using STATISTICA 12 (StatSoft Inc. 2013).

Results and discussion

Biochemical and phenotypic validation of GFP-labeled *Vibrio coralliilyticus*

Biochemical and phenotypic profiling indicate that GFP insertion did not affect the growth dynamics or proteolytic activity of the labeled *V. coralliilyticus* strain. No

significant differences in bacterial growth curves were
detected between GFP-labeled and WT strains of *V. co-
ralliilyticus* over the 96-h trial ($F_{1,2} = 0.004$, $P = 0.96$;
Fig. 1a). The azocasein protease activity assay (Windle and
Kelleher 1997) also indicated no significant loss of prote-
olytic activity following GFP insertion. While the prote-
olytic activities of both the GFP-labeled and WT strains of
V. coralliilyticus were lower than the trypsin positive
control ($P < 0.00$), there was no significant difference in
protease activity between the two strains ($P = 0.34$;
$F_{2,9} = 38.69$, $P = 0.0004$; Fig. 1b).

The mini-Tn7 transposon system used in this study was
highly effective for site-specific tagging of Gram-negative
bacteria, as it inserts a single copy of the GFP gene within a
neutral chromosomal site (Bao et al. 1991). This targeted
insertion ensures that the GFP gene will be passed on to
successive generations and also reduces the probability of
unintentional alterations to the host phenotype (Lichten-
stein and Brenner 1981). The most comprehensively de-
scribed virulence factor for *V. coralliilyticus* involves the
production of a zinc metalloprotease that causes rapid in-
activation of photosystem II within corals' endosymbiotic
algae, *Symbiodinium* (Sussman et al. 2009). Unintentional
alteration of the Zn-metalloprotease encoding gene (*vcpA*)
could dramatically alter *V. coralliilyticus* virulence. The
biochemical and phenotypic results presented here
demonstrate that the GFP insertion process did not sig-
nificantly affect the growth dynamics or proteolytic activity
of *V. coralliilyticus*.
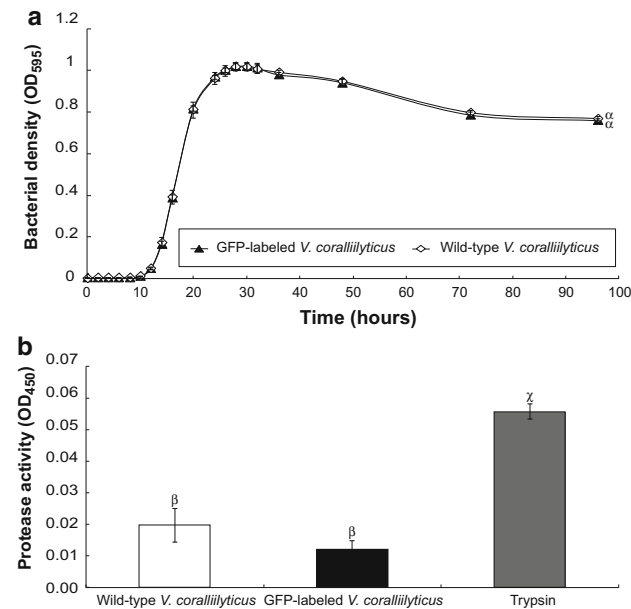
Fig. 1 Comparison of **a** growth rate ($n = 2$) and **b** proteolytic
activity ($n = 4$) between wild-type and GFP-labeled *Vibrio coralli-
ilyticus* P1 strains. *Greek symbols* indicate post hoc groupings
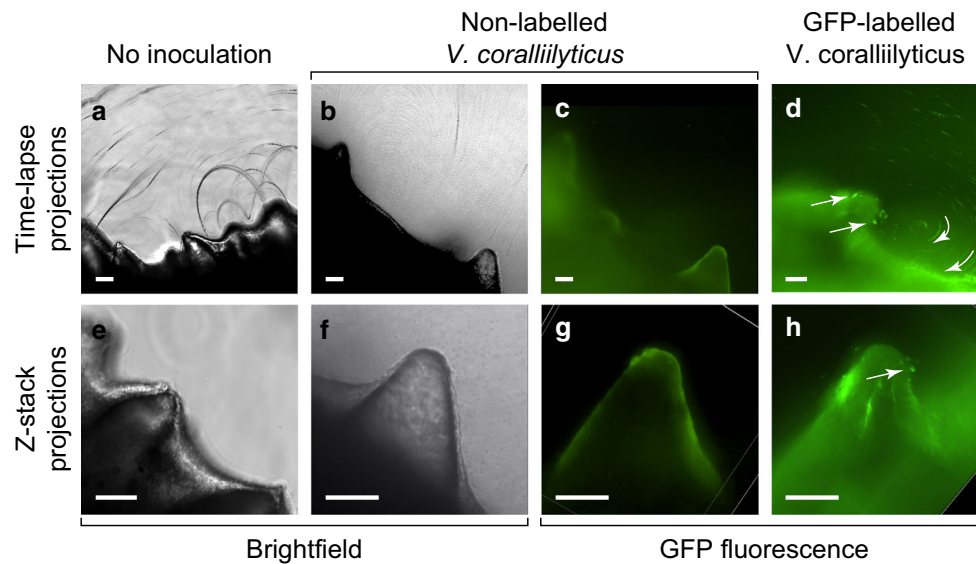(Tukey's HSD $P < 0.05$)

Symbiodinium, coral juvenile, and *Aiptasia* sp. infection
trials

Infection experiments using multiple model systems, in-
cluding *Symbiodinium* algal cells, juveniles of the coral *A.
millepora,* and adults of the sea anemone *Aiptasia* sp.,
demonstrated that GFP insertion did not significantly affect
virulence of the GFP-labeled *V. coralliilyticus* strain. PSII
quantum yields of *Symbiodinium* cells challenged with both
WT and GFP-labeled *V. coralliilyticus* cells were sig-
nificantly reduced relative to the no inoculation control
($P < 0.00$), as indicated by the reduction of PSII yields over
time, but no differences in photo-inactivation were ob-
served between strains ($P = 0.99$; $F_{2,9} = 7064$, $P < 0.00$;
Fig. 2a). Similarly, PSII quantum yields of symbionts
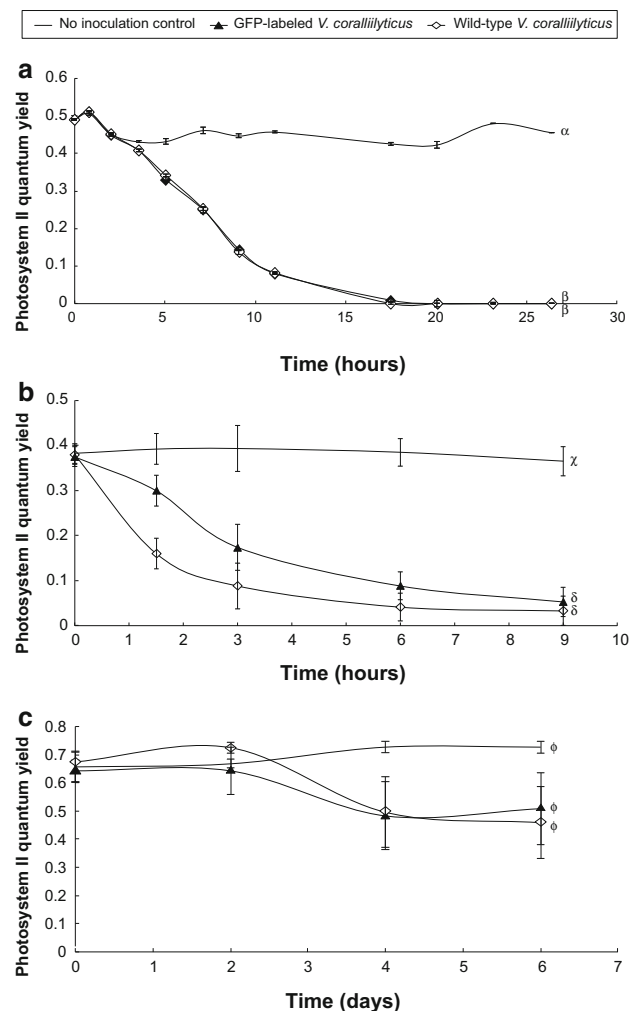
Fig. 2 Photosystem II photosynthetic quantum yield measurements
of *Symbiodinium* in **a** pure culture ($n = 4$), **b** juveniles of the coral
Acropora millepora ($n = 3$), and **c** adults of the sea anemone *Aiptasia*
sp. ($n = 9$) following exposure to GFP-labeled and wild-type *Vibrio
coralliilyticus* P1 bacterial **a** supernatant and **b**, **c** cells and **a–c** no
inoculation controls

Non-labelled
V. coralliilyticus

No inoculation

GFP-labelled
V. coralliilyticus

Time-lapse
projections

Z-stack
projections

Brightfield

GFP fluorescence

Fig. 3 Direct visualization of host/pathogen interactions showing **a–d** time-lapse projections and **e–h** z-stack projections of a *Pocillopora damicornis* coral with (**a**, **e**) its native bacterial community (i.e., no bacterial inoculation), **b**, **c**, **f**, **g** inoculated, non-labeled *Vibrio coralliilyticus* cells and **d**, **h** inoculated GFP-labeled *V. coralliilyticus* cells. Imaging occurred under **a**, **b**, **e**, **f** bright field or **c**, **d**, **g**, **h** GFP fluorescence. *Straight white arrows* indicate GFP-labeled *V. coralliilyticus* cells closely associated with the coral mucus or tissue, and *curved white arrows* indicate cells suspended in ciliary-driven flow fields. *Scale bars* denote 25 μm

within juvenile *A. millepora* challenged with both *V. coralliilyticus* strains were reduced relative to the no inoculation control treatment ($P < 0.02$), and there was no significant difference between the two strains ($P = 0.50$; $F_{2,6} = 13.78$, $P = 0.006$; Fig. 2b). Although neither bacterial strain resulted in significantly reduced PSII quantum yields in *Aiptasia* sp. infection trials ($F_{2,23} = 1.16$, $P = 0.33$), mean yields at the end of the 6-d trial were lower for both *V. coralliilyticus* strains (mean ± SE, WT: 0.46 ± 0.14, GFP: 0.51 ± 0.13) than for no-infection controls (0.73 ± 0.02). Importantly, the effect did not differ between WT and GFP-labeled strains (Fig. 2c).

In the field of human health, model systems have yielded valuable insights into host–pathogen interactions and environmental drivers of disease. Surrogate models like the ones used here are likely to be similarly useful for disentangling complex host–pathogen–environment interactions within the multi-organism coral holobiont system. Use of model systems will also minimize the extraction of protected and vulnerable species from reef sites for experimental work. Using three model systems (i.e., cultured *Symbiodinium*, juvenile *A. millepora*, and adult *Aiptasia* sp.), we demonstrate that *V. coralliilyticus* virulence was not impacted by GFP insertion.

Host–pathogen visualization in situ

Real-time imaging of coral fragments in the presence of *V. coralliilyticus* demonstrated that the insertion of GFP

enabled tracking of the pathogen through the surrounding seawater and on the host surface. Video microscopy captured cells in motion on and around the corals. The tracks of bacteria from these videos are presented as time-lapsed projections (Fig. 3a–d; Electronic Supplementary Materials, ESM, Videos S1, S2, and S3), while the spatial distribution of individual bacteria in association with the host surface was obtained by imaging a given field of view at multiple depths (z-stacks; Fig. 3e–h; ESM Videos S4, S5, and S6). Corals create strong cilia-driven flows that have vortical features stirring the boundary layer (Shapiro et al. 2014; Fig. 3a–d; ESM Videos S1, S2, and S3). Flows in these regions can exceed 1 mm s^{-1}, and thus create a dynamic environment that the pathogen must navigate to reach the host's surface. Unfiltered seawater contains an abundant community of microbes and particles. Although phase contrast microscopy allows their visualization and tracking (Fig. 3a, e; ESM Video S4), it does not enable discrimination of one member of the community from another, and it is particularly challenging to differentiate individual cells on the host's surface due to refraction of light by the coral (Fig. 3a–b, e–f; ESM Videos S1 and S4). When the natural bacterial community was amended with $\sim 10^6$ cells ml^{-1} of WT *V. coralliilyticus*, the flow fields remained visible using phase contrast microscopy (Fig. 3b, f; ESM Videos S1 and S4), but only the coral itself was visible using epifluorescence microscopy (Fig. 3c, g; ESM Videos S2 and S5). When the GFP-labeled strain (at the same concentration) was introduced instead of the WT, individual pathogen cells were clearly visible, both in the

661

flow field (Fig. 3d; ESM Video S3) and in association with the coral's surface (Fig. 3h; ESM Video S6). In summary, the GFP strain provided a simple and reliable method of imaging interactions between *V. coralliilyticus* and its coral host.

In this study, we have demonstrated stable GFP labeling of the coral pathogen *V. coralliilyticus*, confirmed unaltered virulence in the labeled strain, and employed epifluorescence video microscopy to visualize interactions between the labeled pathogen and the coral host. To the best of our knowledge, this study is the first to demonstrate successful chromosomal GFP labeling and visualization of a known coral pathogen.

The GFP-based system developed here can now be used to investigate the pathogenesis of *V. coralliilyticus*-induced coral disease. We have demonstrated that GFP-labeled *V. coralliilyticus* is a useful tool for tracking this pathogen as it moves around the host and attaches to the surface of corals. Future studies can employ this tool to investigate the influence of environmental factors and host physiology, including temperature stress and physical injury, on infection dynamics. Labeling of potential probiotic bacteria (e.g., with cyan or red fluorescence proteins) would allow simultaneous visualization of pathogen and probiotic bacterial strains and could facilitate the discovery of new mitigation methods to control these diseases. This approach could also be adapted for other coral pathogens (e.g., *Vibrio shiloi*) and is compatible with a variety of visualization techniques (e.g., flow cytometry and fluorometric plate reader-based assays). This GFP *V. coralliilyticus* strain thus provides a new opportunity to unravel the mechanistic underpinnings of coral–pathogen interactions, including insights into the dynamics of pathogen attachment, subsequent exclusion by or entrance into host cells, and the bacterium's ability to proliferate in the host microenvironment.

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