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Antagonism between coral pathogen *Vibrio coralliilyticus* and other bacteria in the gastric cavity of scleractinian coral *Galaxea fascicularis*

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Abstract Scleractinian corals host numerous microbial symbionts with different types of interactions. The gastric cavity of scleractinian coral, as a semiclosed subenvironment with distinct chemical characteristics (e.g., dissolved O_2, pH, alkalinity, and nutrients), harbors a distinct microbial community and a diverse array of bacteria that can be pathogenic or beneficial. *Galaxea fascicularis* is one of the dominant massive scleractinian coral species on inshore fringing reefs in the northern South China Sea. Although the abundance of coral-associated bacteria has been investigated in *G. fascicularis*, less is known about the microorganisms in the gastric cavity. In this study, we specially isolated cultivable bacterial strains from the gastric cavity of *G. fascicularis* collected from Hainan Island using a noninvasive sampling approach. Among the 101 representative bacterial strains, one *Vibrio coralliilyticus* strain, SCSIO 43001, was found to be a temperature-dependent opportunistic pathogen of *G. fascicularis*. The antagonistic activity between the 100 strains and *V. coralliilyticus* SCSIO 43001 was tested using a modified Burkholder diffusion assay. Our results showed that *V. coralliilyticus* SCSIO 43001 inhibits the growth of *Erythrobacter flavus* and *Sphingomonas yabuuchiae*. Additionally, we found that three *Pseudoalteromonas* strains showed moderate to high antibacterial activity against *V. coralliilyticus* SCSIO 43001 and several other coral-associated Gram-negative bacterial strains. These results suggest that competition between the coral pathogen and other bacteria also occurs in the gastric cavity of coral, and *Pseudoalteromonas* strains in the gastric cavity of *G. fascicularis* may provide a protective role in the defense against co-inhabiting coral pathogens at elevated temperature.

Keywords Coral pathogen, *Galaxea fascicularis*, Scleractinian coral, *Vibrio coralliilyticus*

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1. Introduction

Scleractinian corals function as the primary reef ecosystem engineers, constructing the framework and shaping the resource availability for other coral reef-associated organisms (Jones et al., 1994). Despite being one of the simplest metazoans, corals harbor some of the most highly diverse and abundant microbial communities. Manipulation of the coral-associated microbiome was postulated as a key strategy to improve the resilience of reef-building corals (Rosenberg et

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al., 2007; Zilber-Rosenberg and Rosenberg, 2008; Bosch and McFall-Ngai, 2011; Sachs et al., 2011; Bosch, 2013; McFall-Ngai et al., 2013; van Oppen et al., 2015). A recent attempt to mitigate the bleaching of the scleractinian coral *Pocillopora damicornis* when exposed to increased seawater temperature has been successful through the addition of native putatively beneficial microorganisms isolated from coral (Rosado et al., 2018).

Galaxea fascicularis, a species of massive reef-building coral of the family Oculinidae, is widely distributed in the Red Sea, the Gulf of Aden and the Indo-Pacific oceans (Veron, 2000). It is also a representative dominant species on inshore fringing reefs in the northern South China Sea (Yu, 2012; Chen et al., 2013). The diversity and structure of bacterial communities and *Symbiodinium* associated with *G. fascicularis* from the South China Sea were analyzed (Gong et al., 2018); (Li et al., 2013). Using massively parallel sequencing of the 16S rRNA gene, the coral-associated microorganisms of *G. fascicularis* collected from five different locations in the South China Sea were compared and assigned to Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacteroidetes, Cyanobacteria, Thaumarchaeota, and Euryarchaeota (Cai et al., 2018).

Microhabitats found on and within coral structures support diverse microbial communities and influence microbial function within the coral holobiont. Coral tissue, mucus, and skeleton microbiomes differed in richness and microbiome composition (Herndl and Velimirov, 1985; Agostini et al., 2012; Pantos et al., 2015; Liang et al., 2017; Rosado et al., 2018). Although microbes have been isolated from the endolith, digestive tracts and endosymbiotic zooxanthellae, most commonly studied coral-associated microorganisms have been recovered from the coral surface mucus layer (Shnit-Orland and Kushmaro, 2009; Agostini et al., 2012; Shnit-Orland et al., 2012). Coral pathogens such as *Vibrio shilonii*, *Vibrio coralliilyticus* and *Serratia marcescens* have been characterized as causal agents of coral bleaching under elevated temperature (Rosenberg et al., 2007). Indeed, *S. marcescens* and *Vibrio* dominate mucus microcosms under laboratory conditions (Sharon and Rosenberg, 2008; Krediet et al., 2009). When their ability to efficiently use mucus is disrupted, the virulence of the pathogen is attenuated (but not abolished), probably owing to the inability of the pathogen to establish within the surface mucopolysaccharide layer (Krediet et al., 2013).

All corals have a common structure: Two tissue layers enclose a lumen, which forms the gastric cavity. The coral gastric cavity can be described as a semiclosed subenvironment within the coral with distinct chemical characteristics (e.g., dissolved O_2, pH, alkalinity, and nutrients), and it also harbors a distinct bacterial community (Agostini et al., 2012). Earlier studies showed that the gastric cavity of scleractinian coral harbors a high bacterial abundance and a specific bacterial community (Herndl and Velimirov, 1985; Agostini et al., 2012). Bacterial communities residing in the gastric cavity may be either pathogenic or beneficial. However, the microorganisms in the gastric cavity of *G. fascicularis* are much less explored. In contrast to branching corals, *G. fascicularis* has a relatively large polyp and gastric cavity. Taking advantage of this feature of *G. fascicularis*, we designed a noninvasive sampling approach to isolate bacteria from the gastric cavity of each corallite to collect fluid samples. We selected representative strains isolated from the gastric cavity of *G. fascicularis*. One of the isolated strains, *V. coralliilyticus* SCSIO 43001 (hereafter referred to as SCSIO 43001), was found to be a temperature-dependent pathogen of *G. fascicularis*. Three *Pseudoalteromonas* strains demonstrated medium to high levels of extracellular antibacterial activity against SCSIO 43001 and several other coral-associated Gram-negative bacterial strains. These results suggest that three *Pseudoalteromonas* strains in the gastric cavity of *G. fascicularis* may provide a protective role in the defense against potential coral pathogens at elevated temperature.

2. Materials and methods

2.1 Ethics statement

Permits for coral sampling were provided by the Management Office of Sanya National Coral Reef Nature Reserve (China), and the Department of Ocean and Fisheries of Hainan Province.

2.2 *G. fascicularis* collection

Coral and seawater samples were collected in July 2011 from the Luhuitou fringing reef (18°13′N, 109°28′E), Sanya, Hainan province, China. *G. fascicularis* colonies were sampled at a depth of 3–5 m using a punch and hammer. The temperature of the ambient sea water was approximately 27–28°C, the average pH was 8.78±0.01, and the salinity was 34. Triplicate samples of each species were collected. The interval distance of sampling was 0.5 m. All nine samples were washed with autoclaved sea water and then placed in sterile plastic bags. Ambient sea water was collected into sterile plastic bottles and then filtered through a 0.22-μm polycarbonate filter membrane (Millipore). All samples were frozen at ~80°C until DNA extraction.

2.3 Isolation and identification of gastric bacteria

Healthy coral and artificially bleached coral after heat treatment in lab were used for isolating bacteria from gastric cavity. Briefly, a 50 μL syringe with a long metal needle could penetrate into the gastric cavity of each corallite for

collection of fluid samples. To alleviate the possible damage to the coral tissue and skeleton, the needle was carefully placed into the space between the primary and the secondary septa. Approximately 10–20 μL of fluid was collected from each corallite, and the sampled coral remained alive after sampling. Samples were diluted and plated on marine broth 2216 agar plates, and 100 μL of different concentrations of fluid were spread over 15% marine broth 2216 (BD Difco) agar plates. After incubation for 1–2 days at 30°C, bacteria exhibiting unique colony morphology were subcultured for purification under the same growth conditions. Each isolate was amplified from its 16S rRNA gene using the universal eubacteria primers (Weisburg et al., 1991) 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). All the amplified sequences were compared with those available in the EzTaxon Database (Kim et al., 2012). All the strains used in this study (Appendix Table S1, https://link.springer.com) were cultured in marine broth 2216 medium.

2.4 *G. fascicularis* bleaching assay

The *G. fascicularis* fragments used for the beaching assay were from the same coral colony and two fragments were used in each treatment. All fragments were cultured for 7 days after defragmentation before use. Each fragment was placed into a 4 liter container filled with 3 liters of seawater and the previously described culture conditions (Ushijima et al., 2014) (Ushijima et al., 2012) with the following modification. SCSIO 43001 was added at a final concentration of 5×10^6~1×10^7 CFU mL^{-1} to 3 liters tank water. The sterile seawater was replaced and the SCSIO 43001 culture was added at the same final concentration every 12 h. The process was repeated for two days. During the two days, the incubation temperature of the coral was maintained at 26°C. On the third day, the incubation temperature was increased to 32°C, and SCSIO 43001 was no longer added. The sterile seawater was replaced every 12 h, and the incubation temperature was maintained at 32°C for 2 days. Images of the fragments were taken every 12 h. Sterile seawater was used as a negative control.

2.5 Modified Burkholder diffusion assay

A modified Burkholder diffusion assay (Burkholder et al., 1966) was used for screening the strains that had inhibitory activity against the growth of SCSIO 43001. To make the test soft agar plate, 200 μL of SCSIO 43001 (OD_{600}~4.9) was mixed with 20 mL of 2216 agar to reach a final agar concentration of 0.75% in the plate. Then, 10 μL of the overnight culture of each test strain was spotted onto the plates. A strain with an inhibition zone was considered an antagonistic strain. The same soft agar plate without SCSIO 43001 was used as a negative control for this assay. The assay was repeated at least three times independently with two replicates each time.

2.6 Screening the antibacterial activity of the antagonistic bacteria

The supernatants of the overnight cultures (~12 h) were collected by centrifugation at 5000 g for 5 min and then filtered through a 0.22 μm pore membrane filter. Three different proportions (4:1, 2:2, and 4:3) between the level of sterile marine broth 2216 and the cell-free supernatants of *Pseudoalteromonas* spp. SCSIO 43201, SCSIO 43202 and SCSIO 43203, respectively, were used to culture SCSIO 43001. Strain *Halomonas meridiana* 214, which does not inhibit the growth of SCSIO 43001 using the modified Burkholder diffusion assay, was used as a controlled strain. Then, the growth of SCSIO 43001 was measured in 96-well plates using a microplate reader set at 595 nm. The methods of culturing the test strains with the supernatants of SCSIO 43001 were as mentioned above.

2.7 Nucleotide sequence accession number

16S rRNA gene sequences of these 101 bacterial strains in Appendix Table S1 are deposited in GenBank under the accession numbers MK801563–MK801663.

3. Results

3.1 Noninvasive sampling of gastric bacteria from *G. fascicularis*

G. fascicularis has relatively large polyps, and the average diameter of the corallite of adult polyps (>12 septum) is 2–4 mm. This feature allows us to use a noninvasive approach to obtain gastric bacteria from live *G. fascicularis*. When removed from seawater, the top edge of the corallites was exposed, and the septa became visible due to the contraction of tentacles (Figure 1a). Then, a 50 μL syringe with a long metal needle was used to penetrate into the gastric cavity of each corallite to collect fluid samples (Figure 1b). To alleviate the possible damage to the coral tissue and skeleton, the needle was carefully placed into the space between the primary and the secondary septa (Figure 1b). Approximately 10–20 μL of fluid was collected from each corallite, and the sampled coral remained alive after sampling. The fluid was diluted and plated on a marine broth 2216 agar plate to isolate indigenous pathogens or probiotics that coinhabit the gastric cavity (Figure 1c). A total of 101 bacterial strains were isolated and identified using the 16S rRNA gene (Appendix Table S1). For the healthy *G. fascicularis*, the cultivable bacterial strains were dominated by Proteobacteria,

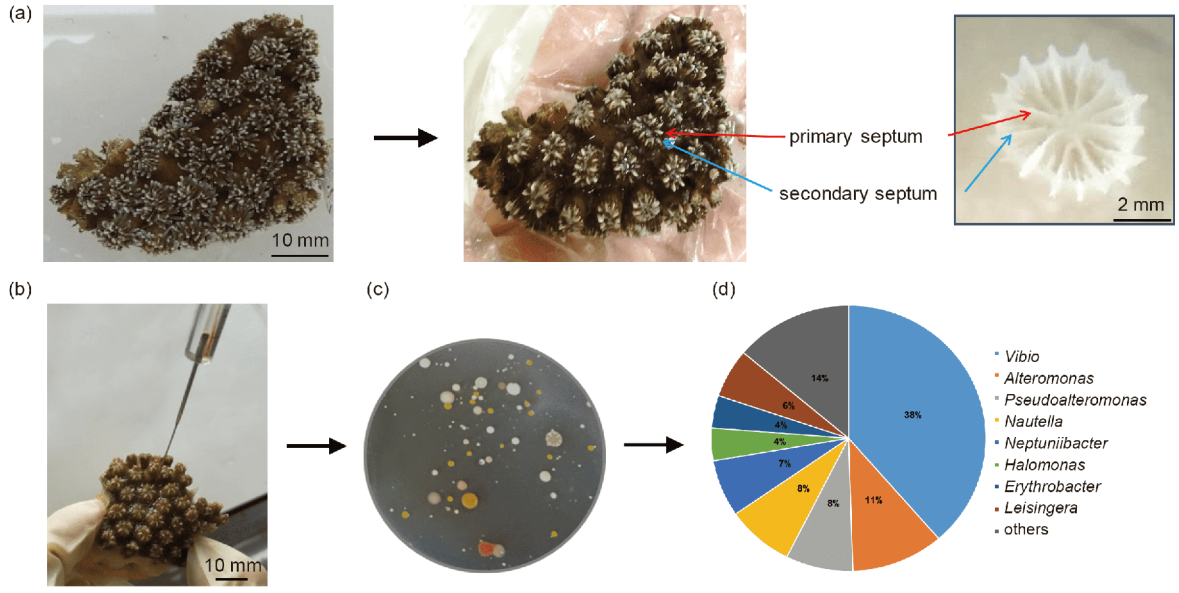

Figure 1 Noninvasive sampling of gastric bacteria from *G. fascicularis*. (a) Fragment of *G. fascicularis* under sea water (left panel) and the corallites exposed (middle panel). The primary septum and secondary septum are shown in the live coral (middle panel) and in the coral skeleton (right panel). (b) Fluid sample was collected by putting the syringe with a long metal needle into the gastric cavity between the primary septum and secondary septum of each corallite. (c) A representative agar plate (2216 medium) shows the diverse morphology of the cultivable bacteria collected from the gastric cavity. (d) The distribution of the 101 cultivable bacterial strains isolated from the gastric cavity was assessed by 16S rRNA sequencing. Strains from different genera are marked by different colors, and the numbers on the pie chart indicate the parentage of each genus.

including those from the genera *Alteromonas*, *Pseudoalteromonas*, *Halomonas*, *Pseudovibrio*, *Erythrobacter*, *Ruegeria* and *Vibrio* (Figure 1d).

3.2 *V. coralliilyticus* accelerated the bleaching of *G. fascicularis*

Vibrio coralliilyticus has been shown to be a temperature-dependent pathogen of scleractinian corals such as *Pocillopora damicornis* (Garren et al., 2016; Rosado et al., 2018), *Acropora cytherea* (Ushijima et al., 2016), *Oculina patagonica* (Rubio-Portillo et al., 2016) and *Mussismilia braziliensis* (Chimetto Tonon et al., 2017). The 16S rRNA gene of one isolate, SCSIO 43001, showed 99.17% similarity to that of *V. coralliilyticus* strain OCN014. Thus, we selected SCSIO 43001 as a candidate pathogenic strain for *G. fascicularis* (Figure 2a).

To explore whether gastric bacteria affect the bleaching of *G. fascicularis* under elevated temperature, we conducted an immersion-based infection assay. To perform the infection assay, SCSIO 43001 was added at a final concentration of 5×10^6 ~1×10^7 CFU mL^{-1} to 3 liters tank sea water and was acclimated at 26°C for 2 days before the temperature was increased to 32°C for 2 days (Figure 2b). The results showed that coral immersed with SCSIO 43001 showed severe signs of bleaching at the coenosarc area (Figure 2c). Moreover, strains that belong to *V. coralliilyticus* were also isolated from the gastric cavity of the bleached coral at elevated temperature (Appendix Figure S1, https://link.springer.com). Thus, SCSIO 43001 is a temperature-dependent pathogen of *G. fascicularis.*

3.3 Antagonism between SCSIO 43001 and other bacteria in the gastric cavity

To identify candidate probiotics, the antibacterial activities of other isolates against SCSIO 43001 were tested by modified Burkholder diffusion assays. As shown in Figure 3a and Appendix Figure S2, three *Pseudoalteromonas* strains showed medium to high levels of antibacterial activity against SCSIO 43001. These strains are renamed *Pseudoalteromonas* sp. SCSIO 43201, *P. elyakovii* SCSIO 43202 and *P. elyakovii* SCSIO 43203 (hereafter referred to as SCSIO 43201, SCSIO 43202 and SCSIO 43203). To test whether the three *Pseudoalteromonas* strains secrete extracellular antibacterial substances, we assessed the growth of SCSIO 43001 in 2216 marine broth supplemented with the supernatant of the antagonists. The supernatant of 12 h cultures collected by filtering through a 0.22 μm pore membrane was then added at different proportions, and the growth of SCSIO 43001 was measured over time. The growth inhibition of SCSIO 43001 was positively correlated with the increasing proportion of supernatant of three *Pseudoalteromonas* strains added to the medium (Figure 3b–3e). Among the three *Pseudoalteromonas* strains, SCSIO 43201 demonstrated the strongest inhibition to SCSIO 43001 and completely inhibited the growth of SCSIO 43001 when the supernatant was added at 75% (Figure 3b).

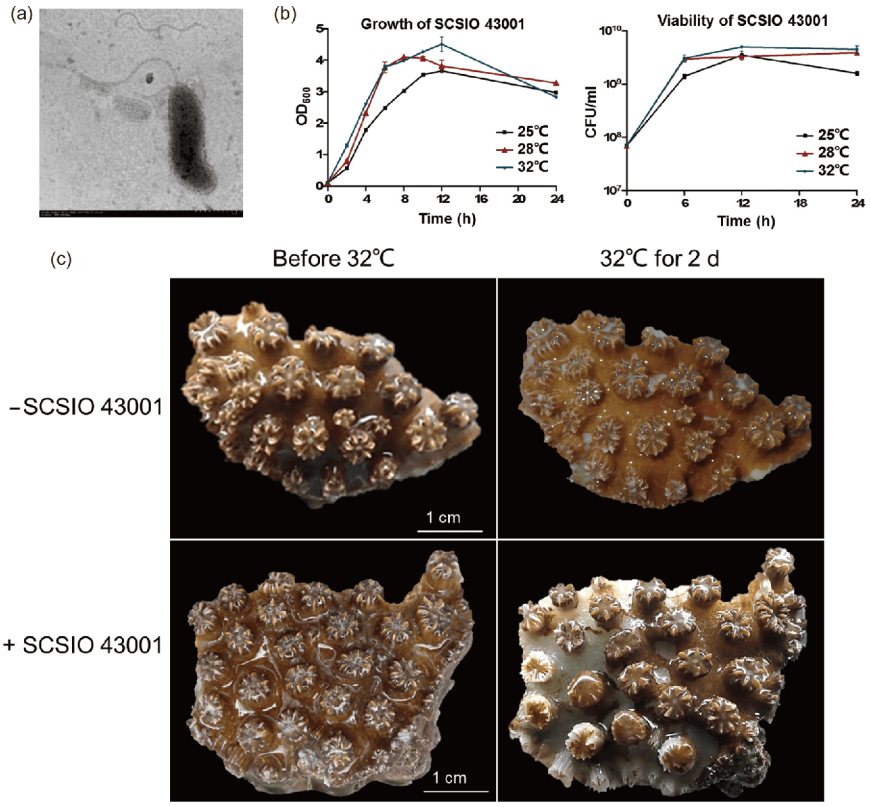

Figure 2 *V. coralliilyticus* SCSIO 43001 accelerated the bleaching of *G. fascicularis*. (a) Scanning electron micrographs of SCSIO 43001. (b) Growth (OD at 600 nm) and cell viability (CFU mL^{-1}) at three different temperatures over time in marine broth 2216. Error bars indicate the standard error of the mean (n=3). (c) Images of the *G. fascicularis* fragments before and after immersion with (lower panel) or without (upper panel) SCSIO 43001. Cultured *G. fascicularis* fragments prior to 32°C treatment (left panel) and under 32°C treatment for 8 days (right panel). Two independent fragments were examined, and only one representative image of each fragment is shown here.

Additionally, we found that SCSIO 43001 can inhibit the growth of four *Erythrobacter flavus* strains and one *Sphingomonas yabuuchiae* strain (Figure 4a). All four *Erythrobacter flavus* produce yellow pigments, and the growth and pigment production was inhibited by SCSIO 43001. Similarly, the supernatant of 12 h cultures of SCSIO 43001 collected by filtering through a 0.22 μm pore membrane was then added at different proportions and the growth of these five strains was measured over time. Except for the *Sphingomonas yabuuchiae* strain, no positive correlation was found between the growth inhibition of the four strains and the amount of supernatant of SCSIO 43001 added (Figure 4b–4f). These results suggest that antagonistic interactions between the opportunistic coral pathogen and other co-inhabiting bacteria in the gastric cavity are common and complex.

3.4 Antagonistic activity of *Pseudoalteromonas* against other strains

Next, we further tested the antagonistic activity of *Pseudoalteromonas* against other bacterial strains isolated from the gastric cavity of coral. As shown in Figure 5a, SCSIO 43201 showed strong inhibition of the other three *Vibrio* strains, *V. parahaemolyticus*, *V. tubiashii* and *V. harveyi*. Both SCSIO 43202 and SCSIO 43203 inhibited *V. parahaemolyticus* and *V. harveyi* but not *V. tubiashii*. Noticeably, the nonpigmented strain SCSIO 43203 showed the strongest inhibition of *V. parahaemolyticus* and *V. harveyi*. Additionally, SCSIO 43201 and SCSIO 43203 inhibited *Alteromonas macleodii* and *Ruegeria conchae*. SCSIO 43202 and SCSIO 43203 inhibited *Erythrobacter flavus* and *Sphingomonas yabuuchiae*.

Furthermore, as one of the dominant genera in the coral-associated microbial community, a high diversity of *Pseudoalteromonas* was also found. Thus, the interplay among different strains of *Pseudoalteromonas* was also tested here. As shown in Figure 5b, the density of the colony formed by SCSIO 43201, SCSIO 43202 and SCSIO 43203 on agar plates was all reduced in the presence of SCSIO 43201, suggesting that SCSIO 43201 might be able to control the abundance of *Pseudoalteromonas* in the coral holobiont. Indeed, cell lysis occurred during the growth of SCSIO 43201 (Figure 5c), suggesting that lytic phages or other agents with lytic activity may provide antagonistic activity against *Pseudoalteromonas* and *Vibrio* strains.

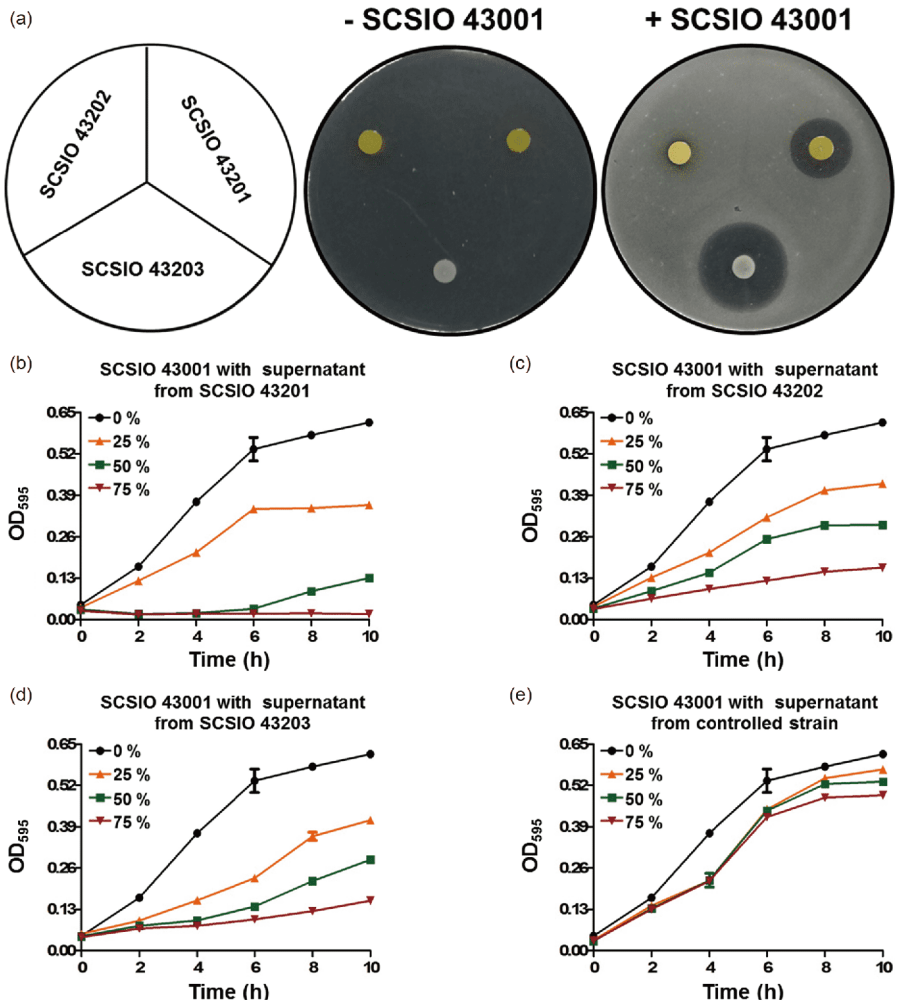

Figure 3 Antagonism between SCSIO 43001 and three *Pseudoalteromonas* strains. (a) A modified Burkholder diffusion assay was used for screening the strains that can inhibit the growth of SCSIO 43001. To make the test soft agar plate, 200 μL of SCSIO 43001 (OD_{600}~4.9) was mixed with 20 mL of 2216 agar to reach a final agar concentration of 0.75% in the plate. Then, 10 μL of the overnight culture of each of three *Pseudoalteromonas* strains was spotted onto the plates. The growth of SCSIO 43001 was measured with the addition of supernatant collected from the overnight culture of SCSIO 43201 (b), SCSIO 43202 (c) and SCSIO 43203 (d). Strain *Halomonas meridiana* 214, which does not inhibit the growth of SCSIO 43001 using the modified Burkholder diffusion assay, was used as a control strain (e). Three different proportions of supernatant of each strain were used, and the growth of SCSIO 43001 was measured in 96-well plates using a microplate reader set at 595 nm. Error bars indicate the standard error of the mean (n=3) in (b)–(e).

4. Discussion and conclusions

In this study, we demonstrated that three *Pseudoalteromonas* from the gastric cavity of coral *G. fascicularis* holobionts are antagonists against SCSIO 43001 and coral-associated Gram-negative bacterial strains. Strains of *Pseudoalteromonas* are widely spread in various marine environments and are commonly found to be associated with eukaryotic hosts, including corals. Many *Pseudoalteromonas* strains commonly produce a variety of biologically active extracellular compounds, including pigmented secondary metabolites, extracellular enzymes and extracellular polysaccharide (EPS) (Kalinovskaya et al., 2004; Bowman, 2007; Yang et al., 2007; Feher et al., 2010; Vynne et al., 2011; Yu et al., 2012). Pigments are organic compounds that confer characteristic colors to living tissues and are usually involved in vital processes (Liu and Nizet, 2009; Soliev et al., 2011). The *Pseudoalteromonas* genus has been divided into two clades based on differences in pigment production. It has been suggested that pigmented strains produce more active compounds. Strains of SCSIO 43201 and SCSIO 43202 produce yellowish and orange-like pigments, while SCSIO 43203 is a nonpigmented strain. Our results showed that the nonpigmented strain also has a high level of antibacterial activity.

Among the three *Pseudoalteromonas* strains, one strain, SCSIO 43201, demonstrated the strongest inhibition against SCSIO 43001 but also demonstrated the broadest range of antimicrobial activity among the 100 tested strains. In particular, SCSIO 43201 can inhibit the growth of strains of

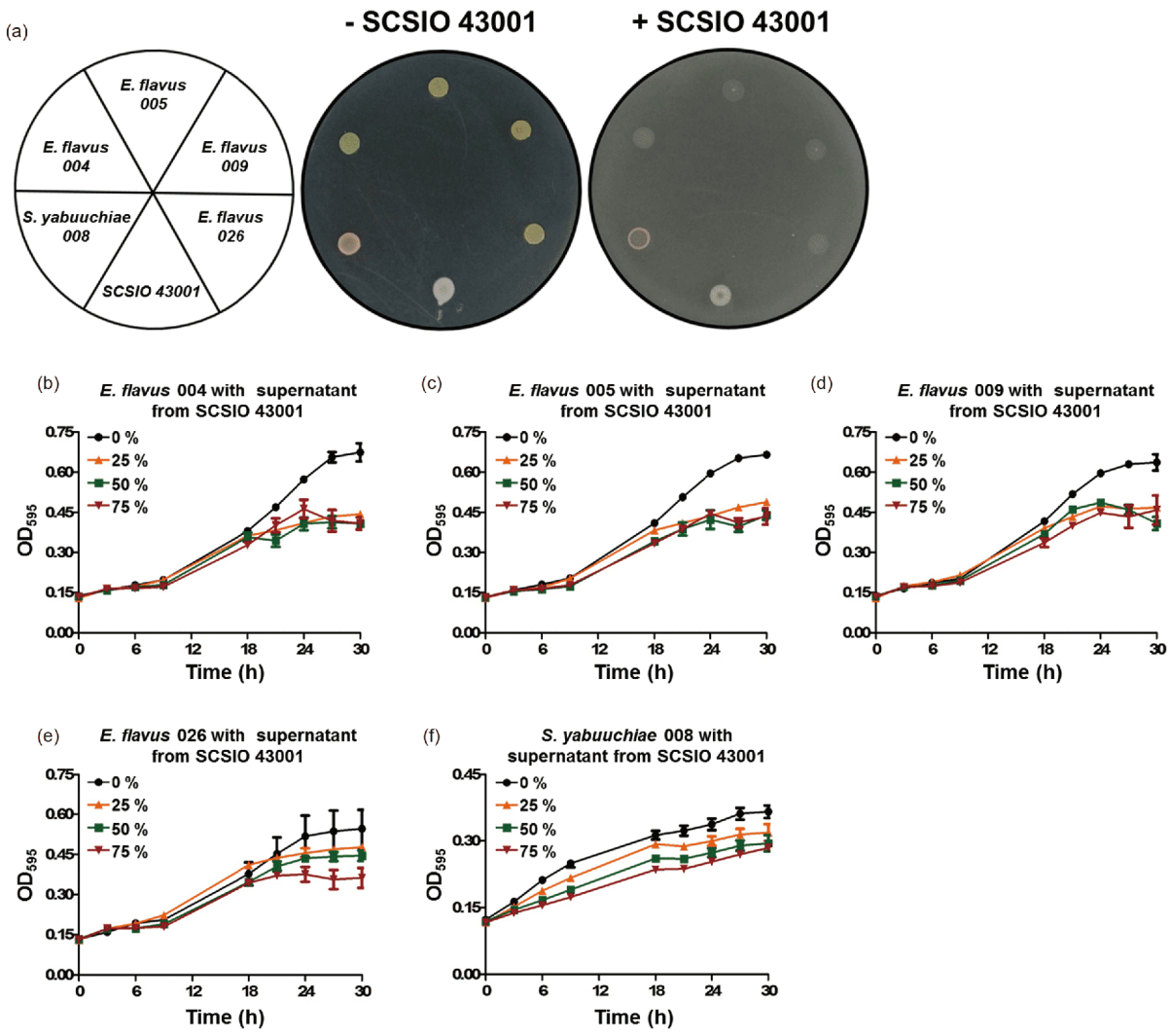

Figure 4 SCSIO 43001 inhibits the growth of *Erythrobacter flavus* and *Sphingomonas yabuuchiae*. (a) A modified Burkholder diffusion assay, as mentioned above, was used to screen the strains that were inhibited by SCSIO 43001. Ten microliters each of *E. flavus* and *S. yabuuchiae* strains were spotted onto the plates. Growth of *E. flavus* 004 (b), *E. flavus* 005 (c), *E. flavus* 009 (d), *E. flavus* 026 (e) and *S. yabuuchiae* 008 (f) was measured with the addition of supernatant collected from the overnight culture of SCSIO 43001. Three different proportions of the supernatant of SCSIO 43001 were used, and the growth of the test strains was measured in 96-well plates using a microplate reader set at 595 nm. Error bars indicate the standard error of the mean (n=3) in (b)–(f).

Alteromonas, *Ruegeria* and *Pseudoalteromonas*. It has been recently reported that strains of *Ruegeria* sp. isolated from *G. fascicularis* can also inhibit the growth of *V. coralliilyticus* (Miura et al., 2019). The genus *Ruegeria* contains a wide variety of bacteria that can produce various bioactive molecules, including quorum-sensing molecules (Sonnenschein et al., 2017), cyclic dipeptides (Mitova et al., 2004), and small noncoding RNAs (Rivers et al., 2016). A recent attempt to mitigate the bleaching of the scleractinian coral *Pocillopora damicornis* when exposed to increased seawater temperature has been successful through the addition of five *Pseudoalteromonas* strains isolated from coral (Rosado et al., 2018). Due to the strong inhibition of both coral pathogens and putative beneficial bacteria, it remains to be determined whether SCSIO 43201 can provide benefits to *G. fascicularis* and improve the resilience of *G. fascicularis*.

Consistent with previous reports (Kim, 2006; Littman et al., 2010), we also observed a shift towards *Vibrio* strains in the cultivable bacteria in the gastric cavity of *G. fascicularis* when explored at elevated temperatures (Appendix Figure S2). In this study, we showed that SCSIO 43001 inhibits the growth of *Erythrobacter flavus* and *Sphingomonas yabuuchiae*. Other *Vibrio* strains, such as *V. owensii* and *V. shilonii,* might have antimicrobial activity against other strains co-inhabiting the gastric cavity. Bacteria can eliminate competitors mainly through contact-dependent and contact-independent strategies (Cornforth and Foster, 2015; Stubbendieck and Straight, 2016). The direct contact-dependent strategy is mainly through the type VI secretion system (Basler et al., 2013; Speare et al., 2018), and the contact-independent mechanism includes the secretion of bacteriocins, antimicrobial compounds and chemical sig-

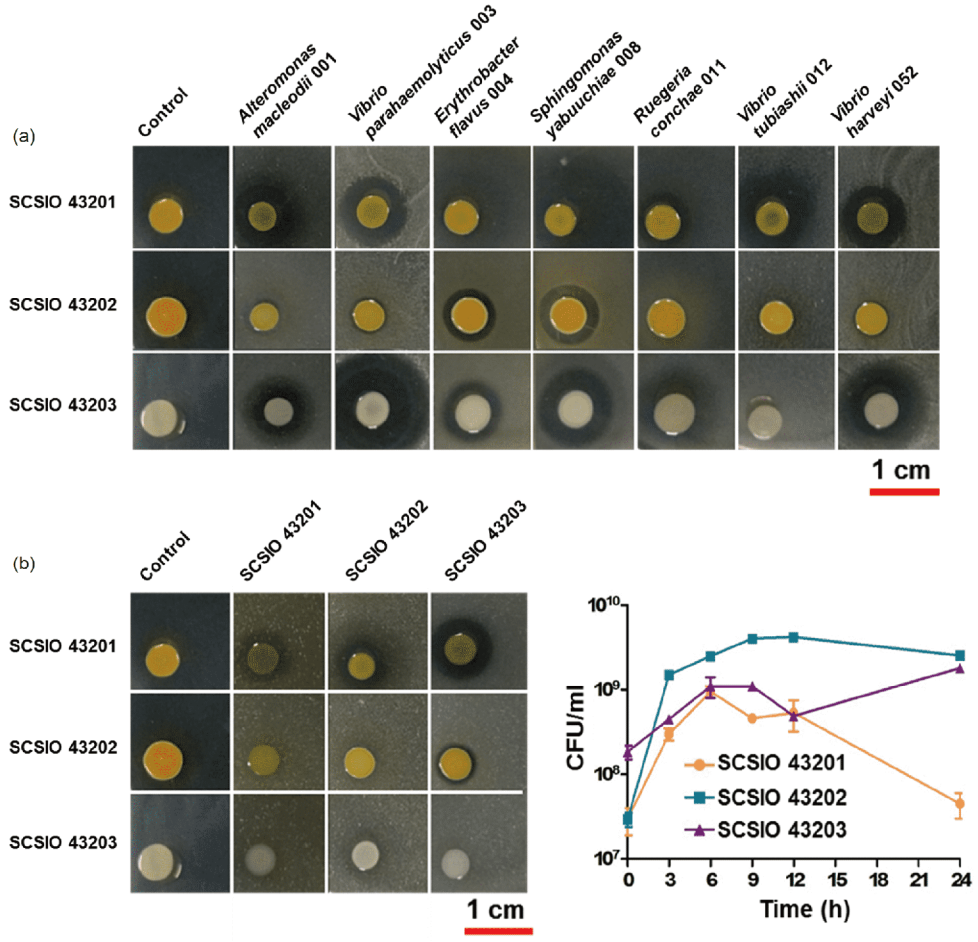

Figure 5 Antagonistic activity of *Pseudoalteromonas* against other strains. Antagonistic activity of the three antagonistic bacteria against other coral-associated Gram-negative bacterial strains (a) and *Pseudoalteromonas* strains (b). Each genus in the upper part of the photos was mixed with 20 mL of 2216 agar to reach a final agar concentration of 0.75% in the plate. Each genus in the left of the photos was spotted onto the soft agar plates. Two independent cultures were conducted, and only one representative image of each strain is shown here. (c) Cell viability (CFUs mL^{-1}) of three *Pseudoalteromonas* strains was calculated over time in marine broth 2216. Error bars indicate the standard error of the mean (n=3).

naling molecules (Chao and Levin, 1981; Hibbing et al., 2010; Tang et al., 2019). We found that *Pseudoalteromonas* strains inhibit SCSIO 43001 through extracellular antibacterial compounds while the inhibition of SCSIO 43001 against other native bacteria is not dependent on the secretion of extracellular compounds. Type VI secretion system is common in vibrios and was found to enable *V. cholerae* to attack members of gut microbiota of mice *in vivo* and promote the pathogen's colonization (Zhao et al., 2018). Further experiments are required to determine whether the antagonistic activity of SCSIO 43001 is dependent on the type VI secretion system.

The species and functions of coral gut bacteria are not well studied, although it has been recognized that distinct microorganisms inhabit in different parts of corals, such as surface mucus layer, coral tissue, gastric cavity and skeleton (Peixoto et al., 2017). It was previously reported that the gut microbiota of corals is similar with free-living communities in the surrounding environment (Ley et al., 2008). However, Agostini and colleges discovered that microbes inhabiting the gastric cavity of *G. fascicularis* are similar with those in the gut of other animals (Agostini et al., 2012). This inconsistent may be caused by the unavailable sampling of the gastric cavity of corals with small polyps. The relatively large polyps of *G. fascicularis* enable us to specifically sampling and study of the microorganisms in the gastric cavity. We isolated bacterial strains belonging to the families of Sphingomonadaceae and Rhodobacteraceae from the gastric cavity of *G. fascicularis*, which is similar with previously reported (Agostini et al., 2012). *Vibrio* spp. were commonly found in surface mucus layer of bleaching corals. It was recently found that *V. coralliilyticus* initially accumulated in the gastrovascular system and then spread to the surface mucus layer (Gavish et al., 2018). We also isolated *Vibrio* spp. from the gastric cavity of healthy and bleaching *G. fascicularis*, which suggests that internal *Vibrio* spp. may proliferate and cause coral disease under heat stress.

Although corals lack an adaptive immune system, it was

previously found that the coral *O. patagonica* can develop resistance to *V. shilonii* (Rosenberg et al., 2007). The coral probiotic hypothesis was proposed to explain these findings (Rosenberg et al., 2007). In this hypothesis, it was supposed that probiotics may be selected during the dynamic change of microbial community under different environmental conditions, which would enable the resistance of corals to specific pathogens. Moreover, the genomes of the coral and its associated microorganisms comprise a hologenome. The hologenome can evolve more rapidly than the coral genome alone through the microbial community shift under changing environments. Therefore, the bacteria in the native coral microbiota that can inhibit pathogens may be an important resource for the selected probiotics. Various coral-associated bacteria were found to show antagonistic activity against *V. shilonii* or *S. marcescens*, including *Pseudoalteromonas* spp., *Roseobacter* sp. and *Exiguobacterium* sp. (Shnit-Orland and Kushmaro, 2009; Rypien et al., 2010; Shnit-Orland et al., 2012; Krediet et al., 2013). However, most of these antagonistic bacteria were isolated form coral surface mucus. In this study, we found that gastric cavity derived bacteria also showed antagonistic activity against *Vibrio* strains. Antagonism is common in microbial communities and contributes to the assembly and longer-term stability of a community (García-Bayona and Comstock, 2018). These antagonistic interactions play key roles in defense and invasion for an ecosystem inhabited by pathogenic or symbiotic microorganisms. Our results showed complex interactions among bacteria in the gastric cavity of *G. fascicularis*, which is important for our current understanding of how the bacteria influence the health of coral and offer insights into manipulation of the microbiota of coral through the planting of beneficial bacteria that are resistant to coral pathogens in the gastric cavity.

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