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NOTE

Interspecific transmission and recovery of TCBS-induced disease between *Acanthaster planci* and *Linckia guildingi*

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ABSTRACT: The susceptibility of the coral-feeding crown-of-thorns starfish *Acanthaster planci* to disease may provide an avenue with which to effectively control population outbreaks that have caused severe and widespread coral loss in the Indo-Pacific. Injecting thiosulfate-citrate-bile-sucrose (TCBS) agar into *A. planci* tissues induced a disease characterized by dermal lesions, loss of skin turgor, collapsed spines, and accumulation of mucus on spine tips. Moreover, the symptoms (and presumably the agent) of this disease would spread rapidly intraspecifically, but interspecific transmission (to other species of echinoderms) is yet to be examined. *Vibrio rotiferianus*, which was previously reported as a pathogen isolated from lesions of experimentally infected *A. planci*, was also recovered from *Linckia guildingi* lesions after several days of direct contact with diseased *A. planci*, demonstrating disease transmission. However, all *L. guildingi* fully recovered after 31 ± 16 d. Further studies are in progress to understand the ecology of *Vibrio* infection in *A. planci* and the potential transmission risk to corals, fishes, and other echinoderms to evaluate whether injections of TCBS could be a viable tool for controlling *A. planci* outbreaks.

KEY WORDS: *Acanthaster planci* · Disease transmission · *Vibrio rotiferianus* · Harveyi group · *Linckia guildingi* · Crown-of-thorns starfish

INTRODUCTION

Outbreaks of the crown-of-thorns starfish *Acanthaster planci* (L.) have had catastrophic effects on many reefs in the Indo-Pacific (Chesher 1969), and remain one of the major causes of coral loss in this region (Bruno & Selig 2007, Pratchett et al. 2011). During outbreaks, high densities of *A. planci* (up to 1 starfish m^{-2}) can rapidly consume >90% of corals across vast tracts of reef habitat (e.g. Chesher 1969). On Australia's Great Barrier Reef, outbreaks of *A. planci* account for 37% of recorded coral loss since 1995 (Osborne et al. 2011). Similarly, at Moorea, in the central Pacific, outbreaks of *A. planci* caused greater levels of coral loss than both cyclones and climate-induced coral bleaching individually (Trapon et al. 2011). Control of outbreaks of *A. planci* may therefore, be the most immediate and effective mechanism by which to reverse sustained declines in the abundance of live coral cover on the Great Barrier Reef (e.g. Bellwood et al. 2004) and throughout the Pacific (Bruno & Selig 2007).

Previous efforts to control outbreak populations of *Acanthaster planci* have been costly and ineffective.

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At Bootless Bay in Papua New Guinea, for example, divers removed (hand-collected) or killed (injected with sodium bisulphate) almost 10 000 individual sea stars during very extensive control efforts, but were still unable either to prevent extensive loss of live coral or to restrict the spread of starfishes (Pratchett et al. 2009). However, *A. planci* (like many echinoderms) are susceptible to infectious disease (Pratchett 1999), which could provide an option for controlling outbreaking populations of *A. planci* (Rivera-Posada et al. 2011a) or urchins (Miller 1985). The close proximity between starfish during outbreaks would facilitate rapid transmission of potential pathogens, eliminating the need to directly kill or collect each individual starfish. Diseases have also been implicated in abrupt declines of *A. planci* numbers at the end of outbreak events (Zann et al. 1990).

Thiosulfate-citrate-bile-sucrose (TCBS) agar is a selective culture medium that inhibits Gram-positive organisms, suppresses coliforms, and allows selective growth of *Vibrio* spp. Rivera-Posada et al. (2011a,b,c) showed that injection of TCBS broth into *Acanthaster planci* digestive organs could induce systemic dysbiosis and facilitate the growth of opportunistic *Vibrio* spp., leading to rapid mortality of individual starfishes. This disease is characterized by dermal lesions, loss of body turgor, matting of spines, and accumulation of mucus on spine tips (Fig. 1A,B). These signs are consistent with natural instances of disease previously observed in *A. planci* (Pratchett 1999). The pathogenic *Vibrio* spp. can also be transmitted to healthy *A. planci* via direct contact with diseased individuals under certain environmental conditions (Rivera-Posada et al. 2011a). Although the possibility of exploiting diseases in *A. planci* for developing biological control measures is promising, significant testing is needed to ensure that there is no chance of disease transmission to non-target species. There have been several exemplary biological control programs implemented in terrestrial systems, but little is known on the potential risks and effectiveness in marine systems (Secord 2003). The purpose of the present study was to explore the potential of interspecific transmission of putative disease agents from *A. planci* to other common and co-occurring echinoderm species, specifically *Linckia guildingi* (gray starfish).

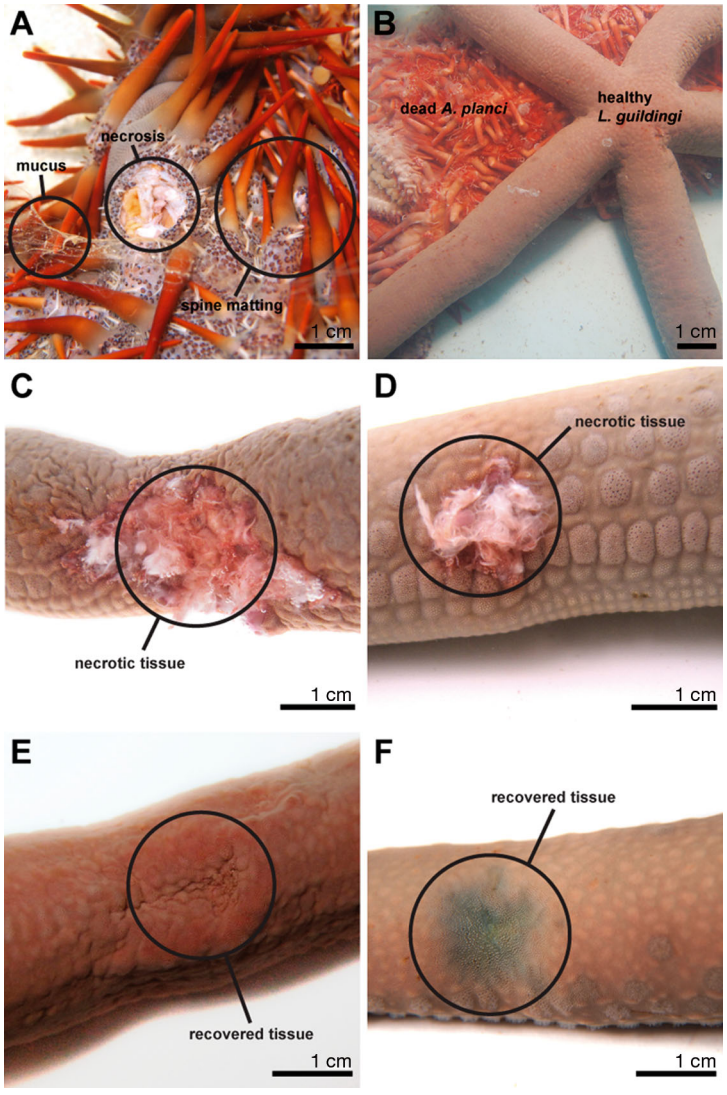

Fig. 1. Thiosulfate-citrate-bile-sucrose (TCBS)-induced disease in *Acanthaster planci*: signs, transmission, recovery. (A) diseased *A. planci* showing loss of turgor, necrotic tissue, mucus, and matting of spines; (B) healthy *Linckia guildingi* in contact with diseased and dead *A. planci*; (C, D) lesions on *L. guildingi* after direct contact with diseased *A. planci*; (E, F) recovered lesions on *L. guildingi*. Scale bar = 1 cm

MATERIALS AND METHODS

To test the possibility of interspecific transmission, seemingly healthy individuals of Guilding's starfish *Linckia guildingi* were placed in direct contact with diseased individuals of crown-of-thorns starfish *Acanthaster planci*. Disease in individual *A. planci* was induced through injection with TCBS, following Rivera-Posada et al. (2011a), which causes proliferation of naturally occurring bacteria (rather than directly introducing any new putative disease pathogens) (Fig. 1A). A total of 5 *L. guildingi* were

placed in individual containers with a diseased *A. planci*, while a further 5 *L. guildingi* starfish were placed in containers in direct contact with healthy *A. planci* to serve as controls. Experiments were performed at the University of Guam–Marine Laboratory with flow-through seawater at ambient conditions (mean temperature = 28°C, pH = 8.3, salinity = 34.5 ppt) for 60 d. All study organisms were collected at Haputo Point, Guam, USA (13° 34′ 14.16″ N, 144° 49′ 18.12′ E).

Bacterial density was monitored every 8 h for 2 d by collecting water and mucus samples, which were appropriately diluted and plated on TCBS plates. Colony-forming units (CFUs) were counted after 24 h incubation at 28°C. Dead and decomposing *Acanthaster planci* were removed from the tanks after 4 d. To identify putative pathogens, lesions on *Linckia guildingi* were swabbed and plated on TCBS agar and DNA was extracted from 4 isolates. Comparable studies were also conducted for *A. planci*, as described in Rivera-Posada et al. (2011a,c). All polymerase chain reaction (PCR) amplifications were performed in a BIORAD 170-9701 PCR thermocycler. PCR reactions (20 µl) contained approximately 20 ng of genomic DNA, 1× PCR buffer (Tris·Cl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$; pH 8.7) (Qiagen), 0.5 µM of each primer, 200 µM dNTPs, and 0.5 units of *Taq* DNA polymerase (Qiagen). The first cycle was preceded by initial denaturation for 15 min at 95°C. Each cycle (30 cycles) consisted of denaturation for 1 min at 95°C, annealing for 1 min at 50°C, and extension for 1 min at 72°C. The last cycle was followed by a final extension step for 7 min at 72°C. Finally, PCR products were visually inspected in ethidium bromide-stained 1% agarose gels against known size standards to verify the presence of amplicons of the expected sizes. PCR products were thereafter purified and sequenced by Macrogen Ltd. using *topA* (topoisomerase I), length 800 nt, VtopA400F (GAG ATC ATC GGT GGT GAT G) and VtopA1200R (GAA GGA CGA ATC GCT TC-GTG) primers (Sawabe et al. 2007).

RESULTS AND DISCUSSION

There were no signs of disease in *Linckia guildingi* starfish collected from the field, nor were starfish placed together with healthy *Acanthaster planci* ever seen to exhibit symptoms of disease. In contrast, 4 out of the 5 *L. guildingi* starfish placed with diseased *A. planci* developed skin lesions within 3 to 9 d (Fig. 1C,D). These lesions could be a result of the lysis of connective tissues induced by opportunistic bacteria as shown in histopathological analyses of diseased *A. planci* (Rivera-Posada et al. 2011b).

Bacterial growth on TCBS medium based on CFUs per milliliter of water sample peaked 32 h after injection, but decreased 8 h later (Fig. 2A). On the other hand, there was an exponential increase in bacterial growth in mucus samples after 24 h which continued to increase for 24 h thereafter (Fig. 2B). The mucus secreted by *Acanthaster planci* after TCBS injection could be a potential pathway for intra- and interspecific transmission of pathogens and partly explains why this induced disease appears to be transmitted only through direct contact (Rivera-Posada et al. 2011a).

Isolates from sampled lesions were identified as *Vibrio rotiferianus*, using the NCBI BLASTN database on the basis of 99% similarity to *topA* gene sequences. This was consistent with topologies based on 16S rRNA, *mreB*, and *topA* analyses previously reported by Rivera-Posada et al. (2011c), which

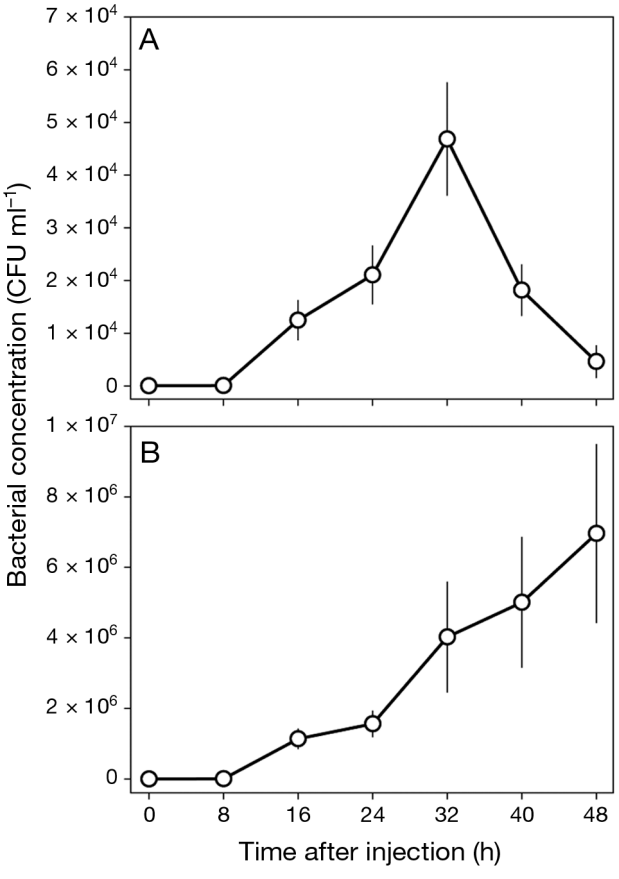

Fig. 2. Bacterial growth after thiosulfate-citrate-bile-sucrose injection: (A) in water samples and (B) in mucus samples. Data are given in mean colony-forming units (CFU) per milliliter (±SE)

described *V. rotiferianus* as an etiological agent isolated from tissue lesions on infected *Acanthaster planci*. Sequences were deposited in GenBank under the Accession Numbers HQ591351, HQ591352, HQ591353, and HQ591354. Nearly all *Vibrio* spp. under the Harveyi group have been reported as causative agents in echinoderm diseases (summarized in Rivera-Posada et al. 2011c). Moreover, pathogenic *V. rotiferianus* was recovered from *A. planci* lesions from mucus travelling from sick *A. planci* to *Linckia guildingi* and from *L. guildingi* lesions. *Vibrio* spp. are well equipped with a series of virulence factors and adaptive response mechanisms such as chemotaxis that greatly influence the infectivity of *Vibrio* spp. (Butler & Camilli 2004). For example, *V. anguillarum* and *V. alginolyticus* undergo positive chemotaxis to mucus collected from fish skin and intestines (Bordas et al. 1998). *V. shiloni*, a coral pathogen, migrates towards coral mucus (Banin et al. 2001), and *V. cholerae* moves into intestinal mucus (Freter & O'Brien 1981).

Lesions in infected *Acanthaster planci* did not recover, and disease progressed, leading to 100% mortality within 24 to 48 h. Rivera-Posada et al. (2011a) also reported mortality in *A. planci* that were in contact with diseased individuals. On the other hand, all *Linckia guildingi* starfish fully recovered (lesions healed) 31 ± 16 d after lesions were first observed (Fig. 1E,F). This disparity in resilience could be attributed to the lower antibiotic properties of *A. planci* compared to other asteroids — showing very weak activity against gram-negative bacteria (Burkholder 1973). Although all starfish were collected in the same area, it is also difficult to determine the level of fitness of each individual, which could also be important in disease susceptibility and recovery. Individuals with higher levels of stress are more susceptible to facultative pathogens (Sutton et al. 1989), such as indigenous *Vibrio* spp.

Disease induction through TCBS injection could be an effective option for controlling *Acanthaster planci* populations at a larger scale. Although this biological control mechanism does not involve the introduction of pathogens, further disease transmission experiments are warranted, especially in corals, carnivorous fishes, and benthic detritus feeders to verify host specificity and vulnerability of non-target species. The present study showed a successful interspecific transmission of disease to *Linkia guildingui*, confirms the pathogenicity of *Vibrio rotiferianus* to echinoderms, and stresses the high risk of applications of TCBS as a control method of *A. planci* outbreaks in the field without more detailed studies of the quorum-sensing mechanisms involved in the induction of disease. It is crucial to take into account that *Vibrio* spp. as opportunistic organisms can evolve and recombine genes under high microbial contact in animal guts or as part of aquatic biofilms, transferring or inducing expression of virulence genes in less-pathogenic or non-pathogenic populations (Thompson et al. 2004). Furthermore, *Vibrio* spp. are ubiquitous in the marine environment, and experimental application of TCBS even in an isolated reef without more detailed studies could have disastrous results on marine ecosystems were no boundaries could limit the spread of disease. This is a portion of on-going work to evaluate whether TCBS injection could be a new tool for the management of *A. planci* outbreaks.

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