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Injection of *Acanthaster planci* with thiosulfate-citrate-bile-sucrose agar (TCBS). I. Disease induction

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ABSTRACT: This is the first report of the successful induction of a transmissible disease in the coral-eating crown-of-thorns starfish *Acanthaster planci* (COTS). Injection of thiosulfate-citrate-bile-sucrose agar (TCBS) culture medium into COTS induced a disease characterized by discoloured and necrotic skin, ulcerations, loss of body turgor, accumulation of colourless mucus on many spines especially at their tip, and loss of spines. Blisters on the dorsal integument broke through the skin surface and resulted in large, open sores that exposed the internal organs. Oedema and reddened digestive tissues and destruction of connective fibers were common. Moreover, healthy COTS in contact with these infected animals also displayed signs of disease and died within 24 h. TCBS induced 100% mortality in injected starfish. There was no introduction of new pathogens into the marine environment. TCBS promoted the growth of COTS' naturally occurring *Vibrionales* to high densities with subsequent symbiont imbalance followed by disease and death.

KEY WORDS: COTS · Corallivores · Culture medium · Infectious disease · Quorum sensing · *Vibrio*

INTRODUCTION

Outbreaks of crown-of-thorns starfishes (COTS) *Acanthaster planci* L., defined as rapid increases in starfish densities to >1500 starfish km^{-2} (Moran & De'ath 1992), represent the most significant biological disturbance on tropical coral reefs (Birkeland & Lucas 1990), resulting in extensive (up to 90%) mortality among reef-building and habitat forming corals (Pratchett 2005, Pratchett et al. 2009). *A. planci* is the principal cause of long-term coral loss at many locations in the Pacific, including Palau, Guam and Fiji (Bruno & Selig 2007, Pratchett 2010). Extensive coral losses caused by outbreaks of *A. planci* also have secondary effects on reef fishes (Sano et al. 1987).

Outbreaks of COTS typically end with rapid population declines, though the causes of these rapid declines are largely unknown (Moran et al. 1985, Moran 1986, Pratchett 2005). In Fiji, the rapid disappearance of *Acanthaster planci* following an extensive outbreak in the 1980s was attributed to disease (Zann et al. 1990). Disease has also been implicated in the mass-mortalities of numerous other echinoderms (Dungan et al. 1982, Lessios et al. 1984, Williams 1986). Pratchett (1999) also demonstrated that tissue removed from sick and dying COTS, collected on the Great Barrier Reef towards the end of an outbreak, can be used to infect other seemingly healthy starfish, indicating that COTS are susceptible to infectious diseases. While there was no attempt to isolate the pathogen involved, Sutton et al. (1988) isolated several potential pathogens, including *Vibrio harveyi, V. tubiashi, V. campbellii, Pseudomonas* and *Moraxella* bacteria, in captive *A. planci* that

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were exhibiting conspicuous signs of disease. However, *Pseudomonas* and *Moraxella* were described as normal flora in coral reef seawater around healthy COTS in subtropical Japan (Okinawa) (Reed et al. 1999). In another echinoderm, *Vibrio harveyi, V. natriegens* and *V. alginolyticus* were described as the causal agents of skin ulceration disease in cultivated juveniles of *Holothuria scabra* (Morgan 2000, Becker et al. 2004). *V. anguillarum* and *Aeromonas salmonicida* also induced the bald sea urchin disease of *Strongylocentrotus purpuratus* (Gilles & Pearse 1986). These pathogenic vibrios coordinate the expression of certain genes in response to their population density through a quorum sensing mechanism (QS), releasing and detecting signal molecules or autoinducers consisting of *N*-acyl homoserine lactones (AHLs). These regulatory systems control various functions, including bioluminescence, conjugative plasmid transfer, biofilm formation, antibiotic synthesis, motility and production of virulence factors in animal, plant and human pathogens (Peters et al. 2003, Fangfang et al. 2008, Park et al. 2008).

Vibrios are usually isolated and cultured in microbiology laboratories by the use of thiosulfate-citrate-bile-sucrose agar (TCBS), which is considered the primary plating medium universally used for the isolation of these bacteria (Kobayashi et al. 1963). This highly selective agar meets the nutritional requirements of *Vibrio* spp., allowing vibrios to compete with intestinal flora (Baron et al. 1994). TCBS components include yeast extract and bacteriological peptone which provide the nitrogen, vitamins and amino acids. Sodium citrate, sodium thiosulfate and ox bile are selective agents, providing an alkaline pH to inhibit Gram-positive organisms and suppress coliforms. An increased pH is used to enhance growth of vibrios, because these organisms are sensitive to acid environments. Sucrose is the fermentable carbohydrate. Sodium chloride stimulates the organism's growth and maintains the osmotic balance of the medium. Sodium thiosulfate is a sulfur source and together with ferric citrate acts as an indicator to detect hydrogen sulfide production. Bromothymol blue and thymol blue are pH indicators and agar is the solidifying agent.

The purpose of this study was to explore a novel method to control COTS through the injection of TCBS agar. This induces a fatal disease that spreads to in-contact COTS. A secondary aim was to identify naturally occurring bacteria in COTS before and after TCBS injections. Moreover, this study examines whether these potential vibrio pathogens would cause symptoms consistent with natural instances of disease observed previously.

MATERIALS AND METHODS

Study sites

The study was undertaken during February–December 2009 at 2 locations: (1) Lizard Island (14° 40′ S, 145° 27′ E) located in the far northern section of the Great Barrier Reef (GBR; Australia) and (2) Haputo Point, northwest Guam (13° 28′ N, 144° 40′ E), in the Western Pacific Ocean.

COTS collection and maintenance conditions

A total of 80 COTS were collected from a range of different depths (3 to 15 m) and from low- and high-density populations. Fifty starfish were from Lizard Island and 30 from Guam. A total of 58 starfish were placed in pairs in 68 l plastic aquariums (Nally bins; 64 cm long × 41 cm wide × 40 cm deep). The other 22 starfish were placed in a large tank (270 cm long × 160 cm wide × 50 cm deep) with constant water flow and an average temperature of 28 to 30°C.

Media cultures

Thiosulfate-Citrate-Bile-Sucrose Agar (TCBS; Oxoid^{R}), Nutrient Agar (NA; Oxoid^{R}) supplemented with 2% NaCl and Marine Broth (MB; Difco^{TM}) (detailed formulas shown in Table 1) were used to culture all organisms of potential significance from the starfish and to determine whether these culture media could induce disease in *Acanthaster planci.* A total of 88 g of the TCBS agar were added to 1 l of reverse-osmosis purified water, heated with permanent agitation to promote the dissolution of the powder but were not excessively heated or autoclaved. This kept the TCBS media in a liquid state for subsequent injection in COTS. MB and NA were prepared following the manufacturer's instructions. All media were stored at 4°C.

Culture media experiments

Five different experiments were carried out.

Expt 1. Nine COTS separated into 3 groups of 3 starfish were used in this experiment. Twelve ml of each medium were injected to individual COTS with a 21 gauge syringe to determine which media culture induced disease. COTS were individually placed in separate aquaria to observe their behaviour, reac-

Table 1. Typical components (g l^{-1}) of culture media and their uses. TBCS: thiosulfate-citrate-bile-sucrose agar

Component	TCBS (g l^{-1})	Nutrient agar (g l^{-1})	Marine broth (g l^{-1})	Function
Yeast extract	5	2	1	Nitrogen, vitamins, amino acids
Peptone	10	5	5	
Sodium chloride	10	5	19.45	Stimulates growth
Agar	14	15		Food additive and solidifying agent
Ferric citrate	1		0.1	Indicator of hydrogen sulfide
Ox bile	8			Selective agents, inhibit Gram-positive organisms and suppress coliforms
Sodium thiosulfate	10			
Sodium citrate	10			
Sucrose	20			Carbohydrate
Bromothymol blue	0.04			pH indicator
Thymol blue	0.04			pH indicator
Lab-Lemco P		1		Protein extract
pH (range)	8.4–8.8	7.2–7.6	7.4–7.8	

tions and the latency period of the disease and time to death.

Expt 2. Fifteen COTS injected with TCBS were used in this experiment; separated into 3 groups of 5 starfish and placed in individual aquaria at 3 different temperatures (26, 28 and 30°C) to investigate whether changes in temperature influenced the bacterial ecology, accelerated the presentation of clinical signs and affected time to death.

Expt 3. Eighteen COTS were placed in pairs into Nally bins; one starfish in each tank was injected with 12 ml of TCBS solution to observe whether the disease induced by TCBS injection could be transmitted to the healthy in-contact COTS.

Expt 4. Sixteen COTS located in pairs in 8 aquariums were used in this experiment. Induction of disease through the injection of 12 ml of TCBS was followed by removal of sick COTS to investigate whether diseases continued to progress without the presence of sick COTS. Once the exposed starfish showed the first clinical signs of mucus on the tip of spines and loss of vertical aspect of spines, the injected COTS were removed and 1 ml of water with mucus was collected to measure bacterial density by the dilution and plating method.

Expt 5. A total of 22 COTS were placed in a large tank at the Marine Laboratory University of Guam (UOGML) at 28.7°C. Two were injected with the TCBS solution to (1) observe whether Guam COTS were susceptible to the disease induced by TCBS injection, (2) determine whether healthy COTS at high densities and in contact would avoid the sick starfish; and (3) investigate whether healthy Guam COTS also become infected with the transmissible disease once they were exposed to sick COTS (outbreak density simulation).

Sample collection and bacterial identification

Microbiology. In order to culture bacteria for later identification, sterile inoculation loops were used to swab the skin surface, dermal lesions and inside the anus of individual starfish. These samples were then streaked on TCBS and NA with 2% NaCl plates and grown for 24 h at 30 to 32°C. Individual colonies from these plates were grown in MB at 30 to 32°C with shaking. Bacterial stocks were stored in liquid nitrogen at −80°C in either MB with 30% (v/v) glycerol or in Microbank™ cryovials (Pro-Lab Diagnostics) for further identification using PCR.

DNA extraction and PCR. Genomic DNA was extracted from overnight MB cultures of 19 COTS bacterial isolates using the Wizard Genomic DNA Purification Kit (Promega®) according to the manufacturer's instructions. Identification of isolates at the level of species was carried out on the basis of the 16S ribosomal RNA gene following PCR amplification and sequencing of this gene as described by Lane (1991), using the primers 27f (AGA GTT TGA TCC TGG CTC AG) and 1492r (GGT TAC CTT GTT ACG ACT T). All PCRs were performed in a GENEAMP PCR System 9700 (Perkin Elmer). PCR reactions (20 µl) contained ~20 ng μl^{-1} of genomic DNA, 1× PCR buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$; pH 8.7) (Qiagen) with a final concentration of 1.5 mM $MgCl_2$, 0.5 µM of each primer, 200 µM dNTPs and 0.5 U *Taq* polymerase (Qiagen). A 5 µl sample of each PCR product was resolved by electrophoresis at 100 V for 30 min in 1% agarose gel, to detect amplicons of the expected sizes and PCR products were sequenced by Macrogen Ltd, Korea.

Phylogenetic analysis. DNA sequences were assembled using the computer software Sequencher 4.9 (Gene Codes). Sequences were manually corrected, trimmed, and finally compared with those of closest relatives available in the GenBank nucleotide database using the BLASTN search algorithm (Altschul et al. 1990). Subsequently, edited sequences were aligned by Clustal_X (Thompson et al. 1997). Phylogenetic trees based on 16S rRNA sequences were constructed with PAUP v.4.0B10 (Swofford 2003) under the neighbour-joining (NJ) algorithm (Saitou & Nei 1987). A total of 19 sequence of the isolates under study and 24 publicly available sequences from type strains of species were used to construct the phylogeny. For the analysis, uncorrected 'p' distance and the Kimura 2-parameter correction (Kimura 1980) were tested. Bootstrap support of phylogenies was calculated on 1000 replicates.

RESULTS

Induction of disease

Expt 1. TCBS was the only media culture that induced death of COTS ~11.5 h post-injection at 30°C. MB induced signs of disease 5 h post-injection but COTS recovered after 10 h. NA broth did not lead to the display of significant signs of disease in healthy individuals.

The characteristic clinical signs of the disease showed by all COTS were discoloured and necrotic skin, ulcerations, loss of body turgor, accumulation of colourless mucus on many spines, especially at their tips and loss of spines. Blisters on the dorsal integument broke through the skin surface and resulted in large, open sores that exposed the internal organs. Oedema and reddened digestive tissues, and lysis of connective fibers and the magnesium calcite skeleton were also common and were observed in infected COTS (Fig. 1).

Expt 2. Changes in temperature significantly delayed the presentation of clinical signs of disease and time to death between 25 and 50%. COTS injected with TCBS and placed at 30°C died within 10 to 14 h; at 28°C died within 14 to 18 h and at 26°C died within 18 to 24 h (Fig. 2). A few starfish lasted longer than others.

Expt 3. Successful transmission to healthy COTS was observed. COTS that were injected with TCBS displayed signs of disease and died within 24 h. In-contact COTS showed similar signs of disease and also died within 24 h (Fig. 3). However, 2 in-contact COTS were exposed to high flow currents without direct mucus contact due to pump pressure problems; one displayed rapid recovery and the other was not infected.

Expt 4. COTS exposed for short periods to sick COTS recovered normal spine positions, reduced mucus production and recuperation was observed after 7 h of solitary confinement. In contrast, COTS with more advanced signs of disease (blisters, open sores) died even when sick TCBS-injected COTS were removed from the aquarium. Increases in bacterial densities ranging between 10^5 and 10^7 colony forming units (cfu) ml^{-1} were observed in these COTS, showing a direct relation with the presentation of disease.

Expt 5. Guam COTS were susceptible to the infectious disease induced by injection of TCBS, and COTS at high densities with open spaces did not avoid sick, in-contact starfish making them more susceptible to disease transmission (Fig. 3). Successful disease transmission was observed and infected starfish died in a similar period compared to those from the GBR (Fig. 3).

Isolates identification

The 16S rRNA gene was successfully amplified from 19 bacterial isolates. BLASTN searches allowed identification of isolates as *Vibrio* spp., *Photobacterium* spp. and *Bacillus* sp. with 99 to 100% sequence identities. In the case of *Vibrio* isolates, phylogenetic analysis based on the 16S rRNA sequences, and not only BLAST searches, was necessary for the identification at species level, due to the high similarities among species for this gene and the presence of multiple unclassified sequences in the databases (Gomez-Gil et al. 2004, Cano-Gómez et al. 2010). For isolates belonging to the *V. harveyi* species group (*V. harveyi*, *V. rotiferianus*, *V. campbellii* and *V. owensii*) the 16S rRNA gene sequence interspecies similarities ranged from 98.9 to 99.6%. The phylogenies offered a more precise identification although low bootstrap support was obtained in numerous nodes (Fig. 4). Strains isolated from sick *Acanthaster planci* were tentatively identified as *V. owensii, V. rotiferianus*, *V. harveyi*, *V. natriegens*, *V. fortis* and *Photobacterium eurosenbergii*. Strains of *Bacillus* sp., *V. fortis, V. rotiferianus, V. owensii* and *P. eurosenbergii* were found in wild healthy animals. Sequences of the 16S rRNA gene were submitted to GenBank (accession nos. HQ44999 to HQ449979; see Fig. 4 for *Vibrio* spp.). Accession numbers for *Bacillus* sp. (isolate: *Beige*, not shown) and *Pseudoalteromonas* sp. (isolate: *Gumar*, not shown) are HQ449959 and HQ449967, respectively.

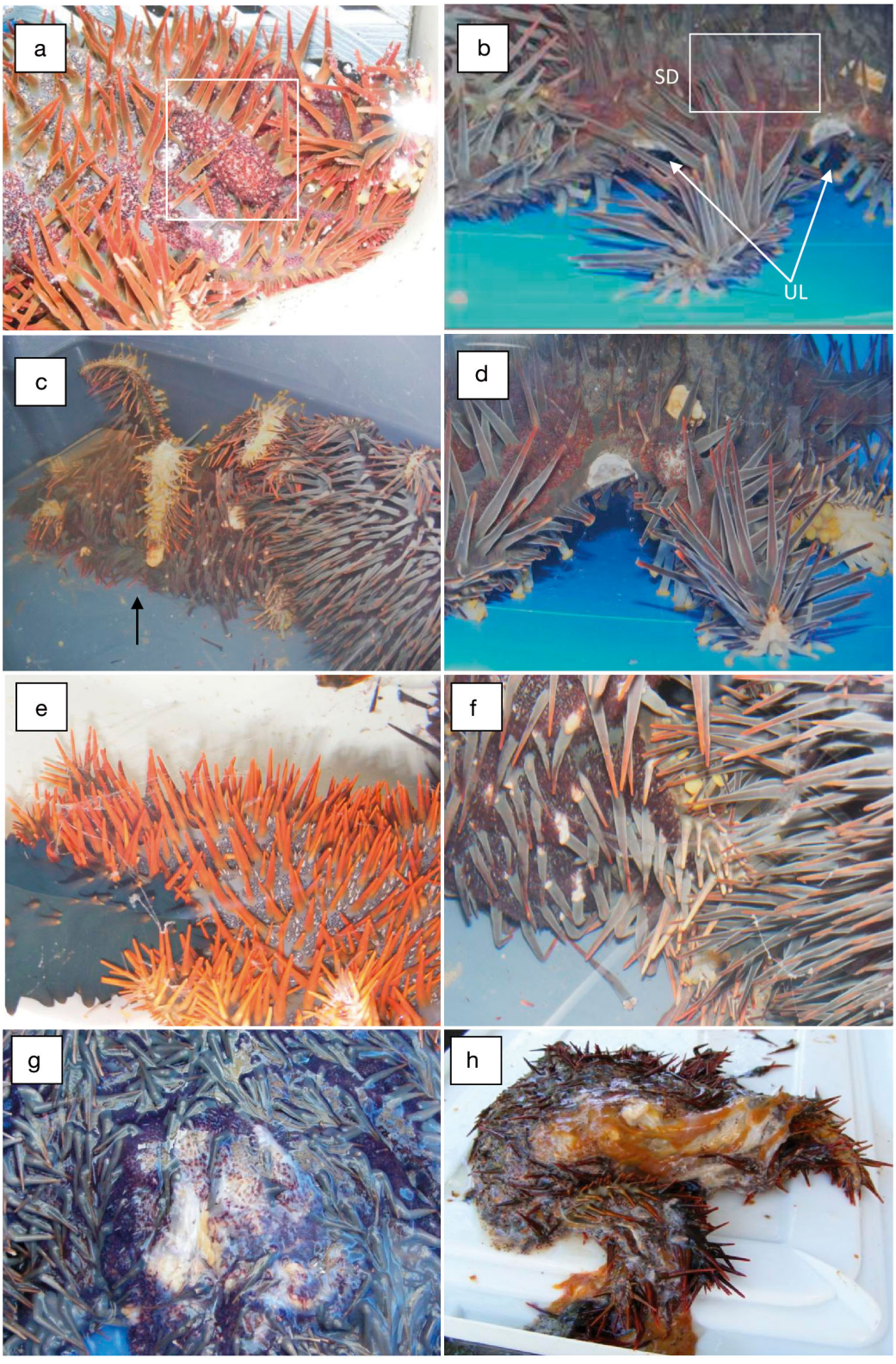

Fig. 1. *Acanthaster planci.* Clinical signs of disease. (a) Loss of skin turgor, blister formation. (b) Ulcerations (UL, white arrows), changes in colour of skin (SD). (c) Loss of a straight orientation and fall of spines (black arrow). (d) Blisters on dorsal surface, ulcerations and exposure of digestive glands. (e,f) Colourless mucus at the top of the spines. (g,h) Destruction of collagen tissues (skin, digestive glands); (H) Death

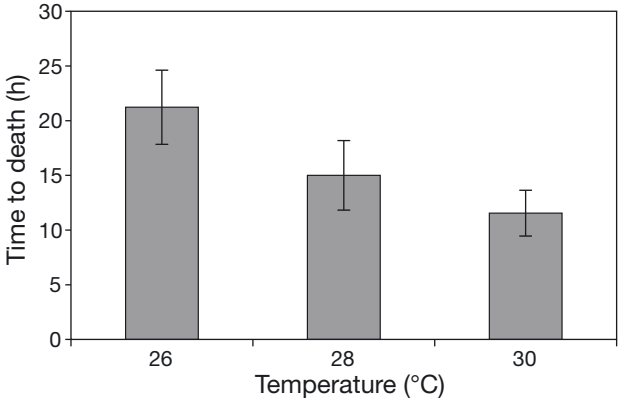

Fig. 2. *Acanthaster planci*. Time to death of crown-of-thorns starfish (COTS) in relation to variations in water temperature (n = 5 COTS per temperature)

DISCUSSION

Method of action

Intestinal flora plays an essential role in health, stimulating the immune system, protecting the host from invading pathogenic bacteria and viruses, supporting digestion and assimilating food (Orrhage & Nord 2000, Guarner & Malagelada 2003). Interactions of typical intestinal bacteria also contribute to stabilization 'eubiosis' or destabilization 'dysbiosis' of the gastrointestinal tract affecting host homeostasis.

Different mechanisms are used by beneficial bacteria to maintain eubiosis within the gastrointestinal tract: (1) secretion of antibacterial substances such as hydrogen peroxide, organic acids and bacteriocins to reduce the number of viable pathogenic organisms, or decrease of bacterial metabolism and toxin production; (2) competition for nutrients necessary for pathogen survival; (3) competition with pathogens to prevent their adhesion to the intestine; (4) enhancement of production of defensive molecules in the host such as mucins; and (5) direct uptake or decomposition of organic matter or toxic material produced by pathogenic bacteria (Brook 1999, Liévin et al. 2000, Guarner & Malagelada 2003). For instance, Jiravanichpaisal et al. (1997) inhibited growth of *Vibrio* spp., *Escherichia. coli* and *Staphylococcus* sp. with *Lactobacillus* sp. in *Penaeus monodon* affected by white spot disease. Conversely, blockage and disruption of beneficial bacteria allow rapid growth of pathogens leading to infection. The greater the imbalance between beneficial and pathogenic bacteria, the greater the symptoms. Under certain conditions, gut microflora can be modified by the substrates which they receive, predominantly through the diet (Bird et al. 2000).

The exact mechanisms that initiate disease with TCBS injection have not been identified. Non-bacterial agents such as viruses or allergic reactions cannot be totally excluded. However, observations using scanning electron microscopy (SEM) did not reveal any viruses (Becker et al. 2004). An allergic reaction to chemicals in the TCBS formulation almost certainly does not initiate a transmissible disease that spreads to healthy in-contact COTS. TCBS, nutrient agar and marine broth formulations share most of their main ingredients. However, nutrient agar and marine broth do not induce death in healthy COTS (control). The main difference between these 3 media is that TCBS also contains ox bile, sodium thiosulfate, sodium citrate and sucrose.

Ox bile, sodium thiosulfate and sodium citrate inhibit Gram-positive bacteria and suppress coliforms inducing dysbiosis of the gastrointestinal tract.

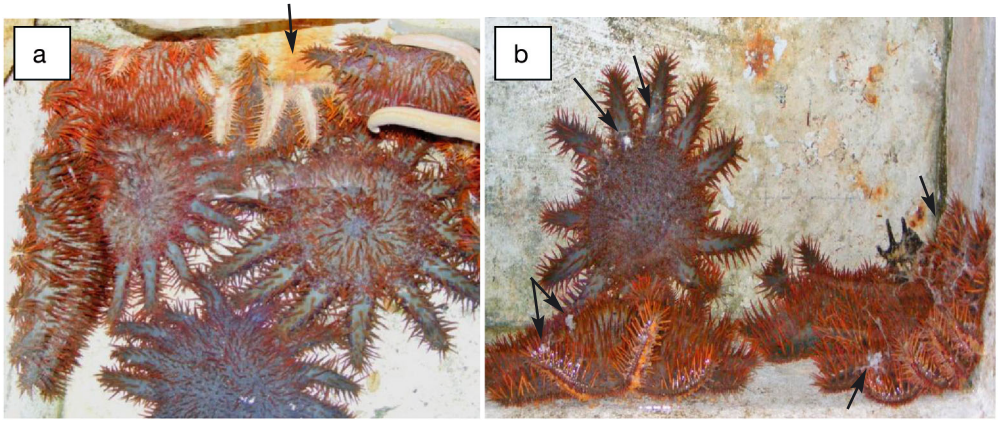

Fig. 3. *Acanthaster planci*. Disease transmission between in-contact *A. planci* at high densities. (a) *A. planci* injected with thiosulfate-citrate-bile-sucrose agar (black arrow) displaying initial signs of disease; there was no avoidance of sick starfish. (b) Infected starfish covered by mucus (black arrows), indicating early signs of successful transmission of disease

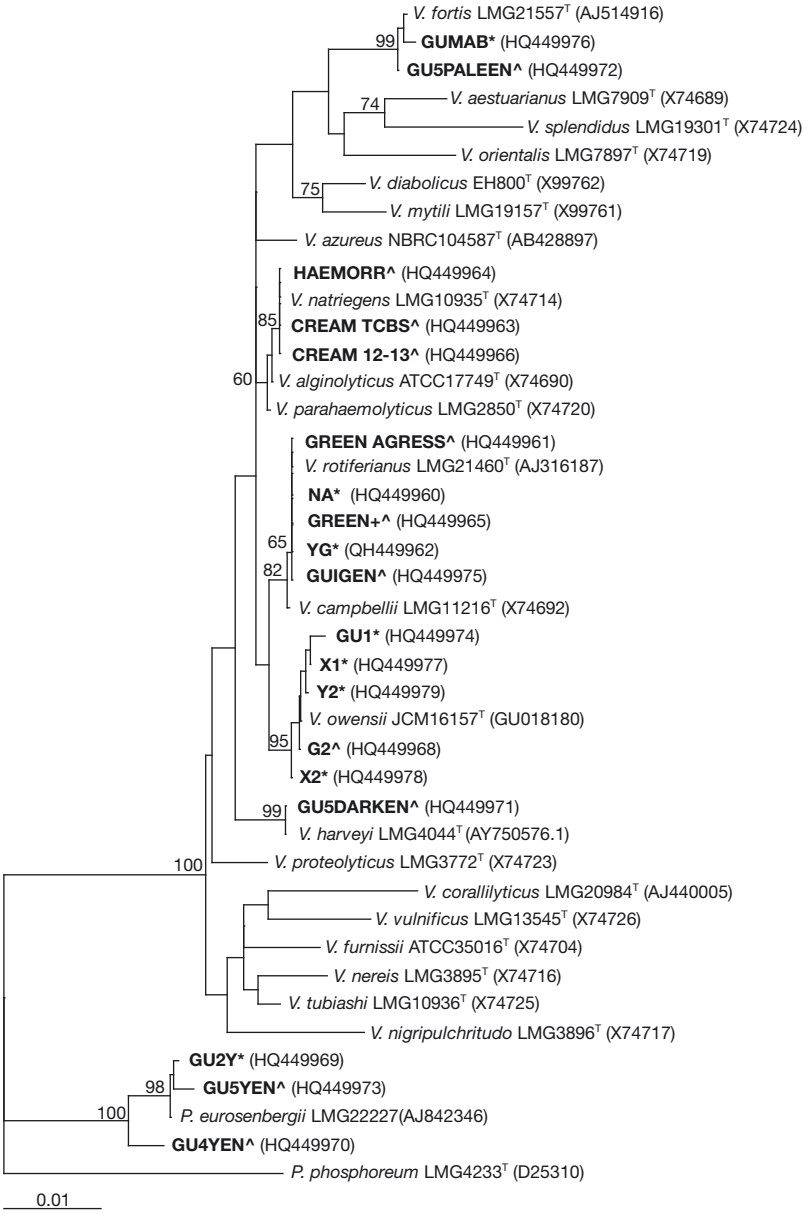

Fig. 4. *Vibrio* spp. Neighbour-joining phylogenetic analysis of *Vibrio* isolates from *Acanthaster planci*, based on partial 16S rRNA gene sequences (1302 nt). GenBank accession numbers are provided in parentheses. *Photobacterium phosphoreum* LMG 4233^T was used as outgroup. Bootstrap support values >50% and after 1000 simulations are shown. Bar shows 1% sequence divergence. 'GU' strains isolated from Guam (USA), *: strains isolated from wild *A. planci*. ^: strains isolated from TCBS-injected moribund *A. planci*

Sucrose supplements the high metabolism of vibrios, promoting rapid growth. We hypothesize that inhibition of competitive bacteria and increase in vibrio cell density activate the quorum sensing (QS) mechanism of the vibrios, turning on virulence factors (Peters et al. 2003) inducing rapid onset of symptoms followed by death.

Pathogenic *Vibrio rotiferianus* (isolates GREEN+ and GREEN AGRESS) and *V. natriegens* (isolates CREAM TCBS and CREAM 12-13) (Fig. 4) were recovered from mucus of infected COTS that spread over healthy in-contact *Acanthaster planci*. *Vibrio* species integrate environmental signalling to modulate behaviour by biasing movements toward more favourable conditions or away from unfavourable environments. Motility and chemotaxis greatly influence the infectivity of vibrios (Butler & Camilli 2004, Larsen et al. 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); *V. coralliilyticus* exhibits chemotaxis towards the mucus of *Pocillopora damicornis* (Meron et al. 2009) and *V. cholerae* moves into intestinal mucus (Freter & O'Brian 1981). Under these conditions and the quorum sensing hypothesis, these migrating vibrios can cause disease in COTS populations by invading in-contact individuals as well as enhancing virulence of normal flora through quorum sensing signalling. Vibrios as opportunistic species 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 or non-pathogenic populations (Thompson et al. 2004).

The features of TCBS coupled with an ideal water temperature, adequate salinity and alkaline pH stimulate *Vibrio* growth to high density, which activates their virulence factors via quorum sensing. Once the COTS immune system fails, skin, ulcerations and exposed organs are colonized by opportunistic bacteria.

Echinoderm body walls consist primarily of collagenous dermis and calcite ossicles. These tissues are known as mutable collagenous tissue (MCT) because they can change stiffness by over an order of magnitude within several seconds. MCT changes are

accomplished by neurally mediated variation in the cation concentration of the proteoglycan matrix. This determines tensile strength, stiffness and viscosity of the body wall, representing a key mechanism involved in many echinoderm functions such as defense against predators, movement, feeding, immune protection against pathogenic bacteria and others (O'Neill 1989, Wilkie 2002). Vibrios as pathogenic agents disrupt the hydroelectric transport of ions (especially Na, K, Cl and HCO_3) inducing tissue structural changes and severe damage (Thompson et al. 2006, Khemiss et al. 2009). Disruption of hydroelectric transport of ions affects the normal echinoderm body wall functions and consequently the subcuticular bacteria symbionts (SCB) located between their ectoderm and surface cuticle. SCB were found in all echinoderm classes and play important roles in defense against bacterial infestation in echinoderms (Burnett & McKenzie 1997). Disruption of transport of ions induced by vibrios at body wall level explains the loss of body turgor (Fig. 1a) and epidermal lesions described in COTS (Fig. 1b,d,g).

Temperature enhancement

Among the numerous environmental factors that influence *Vibrio* spp, temperature had a significant effect in this study. A temperature drop of 4 degrees between experiments reduced the induction of disease and time to death between 25 and 50%, similar to findings of Thompson et al. (2006). Nevertheless, warmer temperatures in combination with other physical factors like alkaline pH, plankton blooms, sunlight and UV intensity can also influence bacterial attachment, growth and multiplication in the aquatic environment, increase the rates of induction and propagation of the CTX phage, and promote viability and culturability of vibrios. For instance, *V. cholerae* remains stable in full sunlight in contrast to enteric bacteria such as *Escherichia coli*, which gives vibrios a clear advantage at tropical latitudes (Mezrioui et al. 1995).

Isolates identification

For species belonging to the Harveyi clade, identification based on 16S rRNA gene analysis is often imprecise (Vandenberghe et al. 2003, Gomez-Gil et al. 2004). Among the Harveyi clade, species of the *Vibrio harveyi* group (*V. harveyi, V. campbellii, V. rotiferianus* and *V. owensii*) have almost indistinguishable phenotypes and genotypes with cases of identical biochemical profiles and ~100% gene sequence identities among different species (Gomez-Gil et al. 2003, Cano-Gómez et al. 2010).

Sequence analysis of the 16S rRNA gene is still essential for bacterial taxonomy (Cohan 2001); however, the resolution power of this molecule is inappropriate in discriminating among closely related species (Kolbert & Persing 1999, Janda & Abbott 2007), such as those belonging to the Harveyi clade. In the present study, the resolution power of the 16S rRNA gene for discrimination of *Vibrio harveyi*, *V. rotiferianus* and *V. owensii* was low with low bootstrap supported tree branches and high inter-species sequence similarities (98.9 to 99.6%). Precise identification was further obscured by the presence of multiple ambiguous nucleotide positions, scored following the degenerate base code. The effect of these positions was evident especially for the closely related clusters of *V. owensii* and *V. rotiferianus*. These ambiguities are related to the presence of 2 chromosomes in some *Vibrio* species (Tagomori et al. 2002), responsible for the existence of multiple divergent copies of the 16S rRNA gene in the same bacterial genome. These arguments support ongoing studies performing biochemical tests and analysis of additional genetic markers for a more precise identification of *V. harveyi*-related species associated with COTS.

Control methods

Attempts to control *Acanthaster planci* outbreaks either by collection of adult individuals followed by burial ashore, by injections of starfish with toxic chemicals affecting other marine organisms or by placing underwater fences are all expensive and time consuming methods only feasible for small areas. Current control methods are ineffective in either eradicating the coral-feeding starfish or preventing further coral mortality. Biological control of populations through manipulation of predators, parasites or pathogens has not been fully investigated (Sutton et al. 1988). Nevertheless, several authors state that predators do not limit the population density of COTS once they reach outbreak levels (McCallum 1987, Moran 1988). Moreover, there are no reports about significant increases in the densities of the major fish predators of COTS (Williams 1986).

Despite more than 3 decades of research and the commitment of millions of dollars, control methods have been unsuccessful as well as costly, time consuming and difficult to accomplish. For example,

divers need to inject chemicals into several areas of each starfish to avoid the starfish simply rejecting the single, injected arm and regenerating. Furthermore, spines are sharp and carry haemolytic toxins (saponins). A puncture wound from a spine is intensely painful and causes oedema, erythema and infection of the surrounding areas. Limiting the exposure of divers to spines by having a propagating epizootic would be a boom to safety. The susceptibility of *Acanthaster planci* to disease could provide an option for controlling population outbreaks. Injection of TCBS culture medium into *A. planci* induces a rapid fulminating disease that is transmitted to in-contact COTS under favourable conditions. Additionally there is no introduction of new pathogens into the environment and it is a rapid and simple procedure with immense economic advantages. However, growth of vibrios enhanced by the TCBS might perhaps have detrimental effects on other reef organisms during a control program. Therefore, further disease dynamics and transmission experiments are warranted, especially in corals, carnivorous fishes and benthic detritus feeders. This is the first part of a series of studies that evaluate whether TCBS injection could be a new tool for management of COTS outbreaks.

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