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Pathogenesis of crown-of-thorns starfish (*Acanthaster planci* L)

Thesis submitted by

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In March, 2012

For the degree of Doctor of Philosophy in Marine Biology and Microbiology, within the
ARC Centre of Excellence for Coral Reef Studies and the School of Veterinary and Biomedical
Sciences, James Cook University

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Jairo Rivera Posada

Abstract

Outbreaks of the crown-of-thorns starfish, *Acanthaster planci* (L.), represent one of the most significant biological disturbances on coral reefs, contributing greatly to widespread habitat degradation across the Indo-Pacific. While the cause(s) of outbreaks are still being debated, an equally important question is what causes population declines at the end of outbreaks. Like all echinoderms, *A. planci* appear to be susceptible to disease, which may explain sudden declines in abundance that have been observed in the wild, as well as providing a unique opportunity to potentially control starfish populations. The purpose of this thesis was to document potentially pathogenic organisms that are normally associated with *A. planci*, as well as describing the pathological progression of artificially-induced disease, following injection of Thiosulfate-citrate-bile-sucrose (TCBS).

Thiosulfate-citrate-bile-sucrose (TCBS) agar is a selective media culture that inhibits gram-positive organisms, suppresses coliforms, and allows selective growth of *Vibrio* bacteria. These bacteria constitute an important part of the bacterial microflora of numerous marine animals, and are also recognized as important pathogens of echinoderms and other estuarine animals. This study showed that injection of TCBS broth into *A. planci* organs induced disease characterized by skin lesions, loss of body turgor, matting of spines, and accumulation of mucus on spine tips. All starfish then died within 24 hours. TCBS broth promoted population growth of naturally occurring *Vibrio* spp., leading to an imbalance in natural symbiont communities. Moreover, diseased starfish often infected seemingly healthy *A. planci* that were either in direct contact or in very close proximity.

To identify potential causative agents of observed disease, and specifically identify all naturally occurring bacteria associated with *A. planci*, starfish were collected from two distinct locations in the western Pacific; Lizard Island (Great Barrier Reef, Australia) and Guam (USA, Western Pacific Ocean). A polyphasic approach, involving histology, scanning electron microscopy (SEM), biochemical profiling using the bacterial API identification system, PCR amplification and sequencing of 16S rRNA, *topA* (topoisomerase I) and *mreB* (rod shaping protein MreB) genes, was used to identify all bacterial isolated. The most significant bacteria isolated from *A. planci* were *V. rotiferianus*, *V. harveyi*, *V. owensii*, *Photobacterium eurosensbergii*, *V. fortis*, *V. natriegens* with sequences identities of 99%-100% for 16S rRNA, *topA*, and *mreB*. Specific bacteria isolated from infected tissues were *V. rotiferianus*, *V. owensii* and *V. harveyi*, which are considered as the most likely causative agents of observed disease.

Histological changes in tissues of *A. planci* following TCBS injection were assessed using conventional and scanning electron microscopy (SEM). Digestive glands were processed and stained with hematoxylin and eosin (H&E) to describe the histological architecture of the intestinal epithelium. Subsequent comparison of healthy versus infected tissues and Gram stains were carried out to confirm bacterial occurrence on infected tissues, characterize the structural changes induced by bacterial communities in COTS tissues and to determine if the histopathological changes of intestinal tissues were consistent with *Vibrio* infection. TCBS injections induced marked epithelial desquamation, hypertrophy and hypersecretion of glandular cells, epithelial cell destruction, pyknosis, reduction of thickness and disorganization of connective tissue and associated nerve plexus, presence of bacterial colonies, irregular eosinophilic foci in glandular cells, brush border disruption, atrophy and detachment of intestinal

microvilli and cell debris in the lumen. All these changes were attributed to a fulminating systemic dysbiosis and were consistent with *Vibrio* infections.

Standard histological procedures used to test for the presence of bacteria are often ineffective for marine organisms. As such, this study developed modified techniques to assess the presence of *Vibrio* bacteria and the preservation of *A. planci* delicate tissues. Detection of *Vibrio* bacteria was improved by the (1) use of short washes before fixation (2) the implementation of short cycles in the processing step; (3) embedding samples in agar prior to automated processing. The use of short cycles also decreased the amount of epithelial desquamation of COTS digestive glands. The study contributes to the standardization of histological techniques and biochemical test (API strips) for partial identification of marine bacteria, ensuring more accurate results, improving performance, enhancing reproducibility and increasing efficiency compared to standard operating procedures.

In order to reverse sustained and ongoing degradation of reef habitat, increasing attention is being given to management and control of *A. planci* outbreaks. Previous control methods, such as hand collecting individual starfish, are extremely labour intensive and often ineffective in either eradicating the coral-feeding starfish or preventing extensive coral loss. As a first step towards assessing whether injections of thiosulfate-citrate-bile-sucrose agar (TCBS) culture medium could be used to eradicate *A. planci*, especially during population outbreaks, we exposed a range of echinoderms to diseased starfish within a closed environment, and also compared naturally occurring bacteria across these echinoderms. *Vibrio rotiferianus*, which was reported as a likely pathogen isolated from experimentally infected *A. planci*, was recovered from *Linckia guildingi*. Moreover, several *L. guildingi* exhibited skin lesions after several days of direct contact with sick *Acanthaster planci*. However, unlike infected *A. planci*, which all died within

48 hrs, all *L. guildingi* starfish fully recovered after 53 days. Further studies need to be carried out to test for cross-infection of *Vibrio* bacteria isolated from sick *A. planci* to corals, fishes and other echinoderms.

To better understand the specific effects of thiosulfate-citrate-bile-sucrose agar (TCBS) on *A. planci*, we tested responses of *A. planci* to individual components of TCBS culture medium. Four out of nine TCBS chemical ingredients tested induced allergic reactions and death in *A. planci*. Peptone 10 g l⁻¹ and oxgall 8 g l⁻¹ induced 100% mortality, while yeast extract and agar induced death in 40 % and 20% of starfish, respectively, 48 h after injection. Peptone was evaluated at three different concentrations (10g, 5g, and 1g l⁻¹). Peptone 10 g l⁻¹ induced 100% mortality, peptone 5 g l⁻¹ killed 60% of injected starfish, and peptone 1 g l⁻¹ induced death in only 20% of starfish, indicating that toxicity of peptone is concentration-dependent. Sodium citrate induced moderate mucus production, but disease did not progress and all starfish completely recovered after 52 h. The remaining chemicals tested, sodium thiosulfate, ferric citrate, mix of sodium thiosulfate + ferric citrate, sucrose and sodium chloride did not produce any kind of clinical signs of disease. This study reported four new components that induced disease and death in *A. planci*. Peptone, oxgall, and yeast are potentially useful in controlling outbreaks because these simple protein extracts can be safer to use compared to previously used noxious chemicals. In addition, lowered concentrations are required to kill *A. planci*, potentially increasing efficiency and effectiveness of established control programs.

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Statement on Sources

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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Jairo Rivera Posada

6th March, 2012

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