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A POTENTIAL PATHOGEN THAT AFFECTS *BRIAREUM ASBESTINUM*

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ABSTRACT

Briareum asbestinum (Anthozoa: Gorgonacea) is a purplish-red gorgonian coral that is commonly found throughout the Caribbean. In the fall of 1998, widespread bleaching, or loss of color due to the expulsion of symbiotic algae called zooxanthellae, occurred along the Florida Keys due to warm water temperatures. At this time, it was noticed that many of the bleached *B. asbestinum* were exhibiting signs of necrosis occurring in patches along the length of the coral colony. These patches of necrosis that occurred along with the bleaching ultimately led to the death of the entire coral organism. Microbiological examination of necrotic tissue resulted in the discovery of a cyanobacterium. However, it is necessary to establish the role of the cyanobacteria in this disease. Therefore, the first goal of this research is to isolate, culture, sequence, and identify this potential pathogen. The second goal is to test whether or not the cyanobacterium is responsible for the death of the *B. asbestinum* along the Florida Keys.

INTRODUCTION

Twenty years ago it was rare to hear a report of a disease affecting corals and coral reefs, but since then, the number of reports has increased dramatically (Santavy and Peters, 1997). Research has revealed the causes of a few of these diseases, and the list of potential pathogens is diverse and may include bacteria, protozoans, fungi, and cyanobacteria (Garrett and Ducklow, 1975; Ramos-Flores, 1983; Rutzler and Santavy, 1983; Kuta and Richardson, 1996). For example, Black Band Disease, which primarily affects corals of the genera *Montastrea*, *Colpophyllia*, and *Diploria*, is actually a microbial consortium consisting of *Phormidium corallyticum*, a cyanobacterium (Rutzler and Santavy, 1983; Taylor, 1983), *Beggiatoa sp.*, a sulfide-oxidizing bacterium, a *Desulfovibrio sp.* sulfide producing bacterium (Garrett and Ducklow, 1975), several other heterotrophic bacteria (Ducklow and Mitchell, 1979), and at least one marine fungus (Ramos-Flores, 1983). Another example is Aspergillosis, which affects *Gorgonia ventalina* and *G. flabellum* (Anthozoa: Gorgonacea), two

types of sea fans. This disease has been shown to be caused by *Aspergillus sydowii*, a terrestrial fungus (Smith et al., 1996). Despite these discoveries, the causal agents of many diseases are still in question (Richardson, 1998), and the role of Man in this increase of diseases cannot be assessed until these questions are settled (Antonius, 1981).

In the fall of 1998, *Briareum asbestinum* (Anthozoa: Gorgonacea) along the Florida Reef Tract experienced a bleaching event where some change in the environment caused the coral to lose their symbiotic algae, or zooxanthellae, leaving the coral tissue white in color. Bleaching can be caused by a wide variety of environmental changes including alterations in temperature, salinity, light intensity, and turbidity (Peters, 1997). The bleaching itself was not unusual, but in addition to this, some coral colonies displayed necrotic patches along the structures. Upon closer investigation of these necrotic colonies, a cyanobacterium was observed growing in the tissue.

Although Feingold observed an unidentified cyanobacterium growing on diseased *Pseudopterogorgia acerosa* along the Florida Reef Tract in 1988, and *Phormidium corallyticum* is known to be a part of the consortium that comprises Black Band Disease (Rutzler and Santavy, 1983; Taylor, 1983), the presence of the cyanobacterium was unusual. Therefore it was hypothesized that the cyanobacterium was causing the death of the bleached *B. asbestinum* although other potential pathogens have still not been ruled out. In order to test this hypothesis, it is necessary to isolate, culture, and identify the cyanobacterium. Furthermore, Koch's Postulate must be fulfilled in order for the hypothesis to be supported (Koch, 1882). The first step in Koch's Postulate requires that microbial samples must be taken from a diseased organism. These samples must be isolated, cultured, and identified in a laboratory. From these cultures, healthy organisms must be inoculated with a potential pathogen in a laboratory setting. At least one healthy individual must not be inoculated to serve as a control. If the inoculated organisms display the same signs of disease seen in the original diseased organisms, another set of microbial samples must be taken from the newly diseased organisms. Again, these samples must be isolated, cultured, and identified. The potential pathogen must again be isolated and cultured from the inoculated organism in order for Koch's Postulate to be satisfied. Although there is not yet enough information collected with this study to neither support nor refute the hypothesis, research to determine the role of the cyanobacterium in this newly observed is ongoing.

METHODS

Branches of several bleached *B. asbestinum* colonies, both healthy and diseased, were acquired from the Florida Keys at the time of the bleaching event. The samples were kept in plastic bags with a small amount of seawater and stored at 4°C to prevent further tissue decay. In order to culture the cyanobacterium, several samples of seawater and coral tissue, some healthy and some containing the cyanobacterium, were taken from each branch and placed into several different media. The media consisted of various combinations of nutrients and trace elements including nitrogen in three concentrations (25, 30, and 35 mg NL^{-1}), 0.01g potassium phosphate, and 1% glucose in either 125 ml sterilized distilled water or 3.2% sterilized seawater. All combinations were kept under constant light provided by a 100 watt bulb and were placed on a slow shaker to provide aeration.

RESULTS AND DISCUSSION

After culturing the organism for several months, the cyanobacterium has been morphologically identified as being part of the genus *Scytonema*. Members of this genus have been known to secrete toxins, which can cause death to the colonized organism. This may be the cause for the patchy necrosis observed in the diseased *B. asbestinum* colonies although the role of the cyanobacterium needs to be further investigated. The DNA of the cultured and isolated cyanobacterium will be amplified using PCR, sequenced, and identified using the 16s ribosomal RNA gene and compared using the GenBank/EMBL database. Once this is accomplished, both bleached and healthy *B.*

asbestinum samples in laboratory aquariums will be inoculated with the cyanobacterium and observed. If the inoculated corals display the same signs as the original disease, the procedures for Koch's Postulate will be followed. If the inoculated corals do not exhibit the same signs as the diseased *B. asbestinum* displayed in the Keys, the procedure will be repeated looking for other potential pathogens. It is likely, however, that the host must be stressed before the pathogen can become established (Harvell et al., submitted).

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