OCCURRENCE OF THE BLACK BAND DISEASE CYANOBACTERIUM ON HEALTHY CORALS OF THE FLORIDA KEYS

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Black band disease (BBD) of corals consists of the migration of a microbial consortium that completely degrades coral tissue while leaving a denuded coral skeleton in its wake. The disease is most common on scleractinian corals (Antonius, 1981; Rützler et al., 1983; Edmunds, 1991), but also has been reported to infect gorgonians (Antonius, 1985; Feingold, 1988). The migration rate of the band is commonly 3 or 4 mm d^{-1}, but can cover a distance of 1 to 2 cm d^{-1}. For scleractinian, reef-building corals which grow on the order of 1 cm in circumference per year (Hudson, 1981), this rate of tissue destruction by BBD can denude entire coral colonies. Coral mortality by black band disease is considered to be an important contributing factor to the observed decline in coral reef health at regional to global scales (Dustan, 1977; Peters, 1984; Williams and Bunkley-Williams, 1990; Porter and Meier, 1992).

The black band consortium is dominated in terms of biomass by a gliding, filamentous cyanobacterium, originally identified as *Oscillatoria submembranaceae* (Antonius, 1973) but reclassified as *Phormidium corallyticum* (Rützler and Santavy, 1983). The band appears black (Fig. 1 top) due to the high concentration of *P. corallyticum*, which contains the light-harvesting red photosynthetic pigment phycoerythrin. Additional microorganisms identified within the band include sulfate-reducing (*Desulfovibrio* spp.) and sulfide-oxidizing (*Beggiatoa* spp.) bacteria (Antonius, 1981), heterotrophic bacteria (Ducklow and Mitchell, 1979), and marine fungi (Ramos-Flores, 1983). Together these microbes physiologically generate vertical microgradients of oxygen (surface) and sulfide (base) within the band which are directly analogous to those found in microbial mats of other benthic aquatic ecosystems (Carlton and Richardson, 1995; Richardson, 1996; Richardson et al., 1997).

The identity of the black band pathogen has yet to be confirmed by tests with axenic cultures. Most members of the consortium have been suspected of initiating the disease. Thus sulfidogenic, sulfide-oxidizing, and heterotrophic bacteria have been implicated (Ducklow and Mitchell, 1979; Garrett and Ducklow, 1975), as has the cyanobacterium (Antonius, 1981; Rützler and Santavy, 1983). Antonius (1988) reported that black band disease occurs specifically after an infection of white band disease. The microbial constituents of white band disease, however, appear to vary among affected colonies (Peters et al., 1983; Ritchie and Smith, 1995) and the identity of the [white band] pathogen(s) is not known. The consensus of several authors (Antonius, 1973, 1981, 1985, 1988; Edmunds 1991; Rützler and Santavy, 1983; Rützler et al., 1983; Taylor, 1983) is that the primary pathogen responsible for black band disease is the cyanobacterium *P. corallyticum*.

The consistent dominance of *P. corallyticum* in active black band supports the hypothesis that it is a required consortium participant, if not the primary pathogen. However, there is little information on its occurrence outside of the black band association. Microscopic examination of phytoplankton net tows (Rützler and Santavy, 1983) failed to reveal *P. corallyticum* filaments in the water column. The only report of a benthic occurrence of this cyanobacterium is that of Taylor (1983) who reported filaments, identified

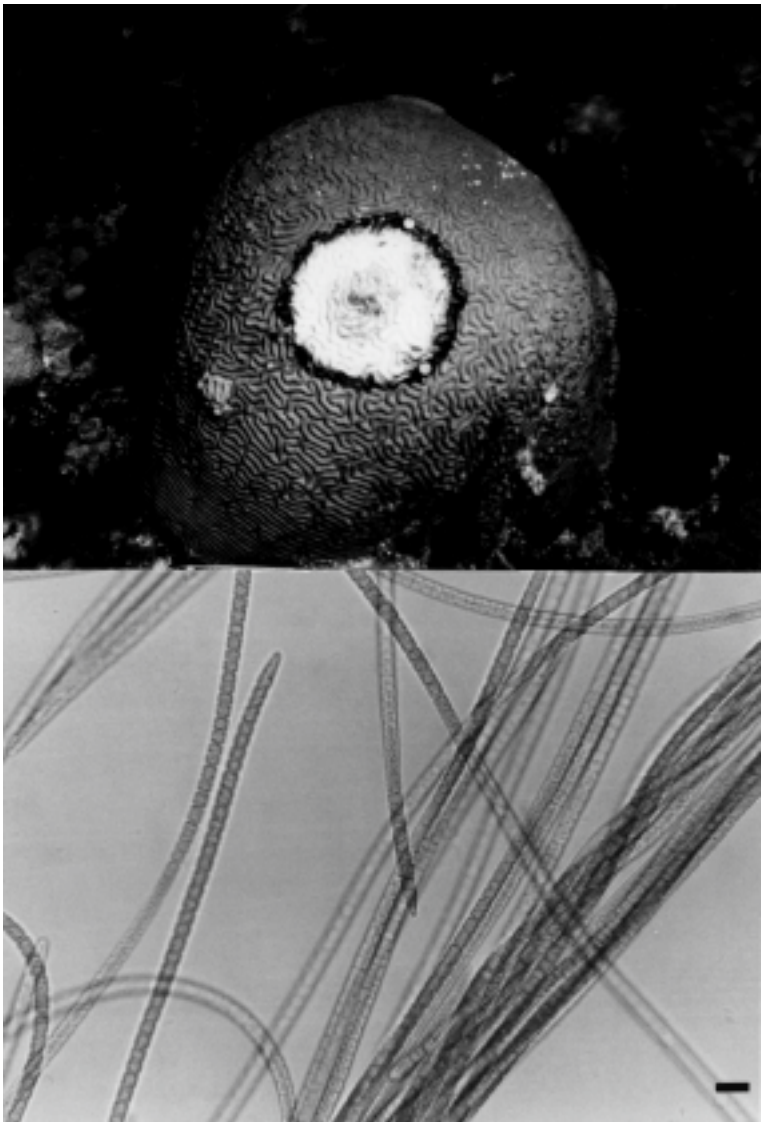

Figure 1. (top) Black band disease on *Diploria labyrinthiformis* at Algae Reef, Key Largo, Florida. Diameter of area denuded by black band is 17 cm. (bottom) Photomicrograph of *Phormidium corallyticum* collected from black band. (Scale = 10 μm)

by light microscopy, from shaded areas under reef limestones not in association with living corals during studies conducted in Florida, the Bahamas, and Jamaica.

In 1994 a survey was conducted of epilithic, epipsammonic, epizoic, and epiphytic microalgal populations on coral reefs of the northern Florida reef tract for free-living *P. corallyticum*, i.e., filaments not in association with BBD. The results presented here document a natural reservoir of *P. corallyticum*.

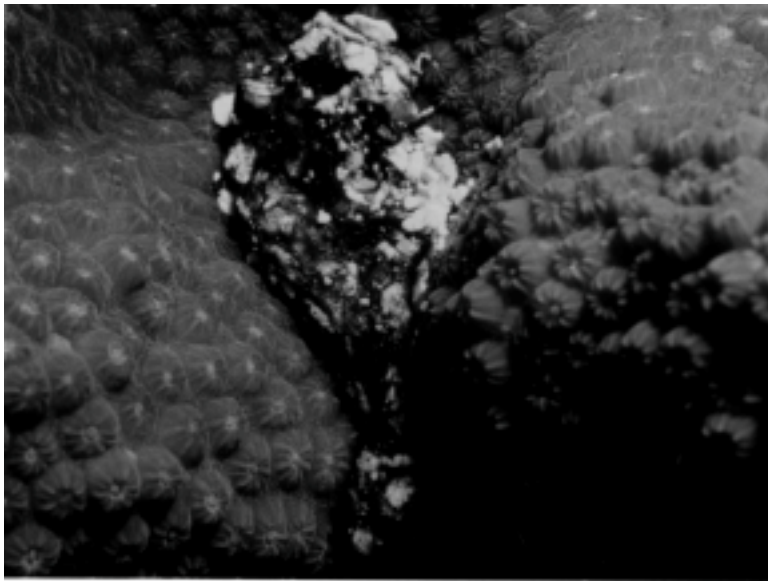

Figure 2. Macrophotograph of mixed cyanobacteria on a sediment patch present on a healthy colony of *Montastrea annularis* (species complex). *Phormidium corallyticum* and *Oscillatoria* spp. were present. The patch was monitored for 17 months (February, 1994 to June, 1995), but never developed into black band disease. (The diameter of retracted coral polyps is 3 mm.)

Materials and Methods

Samples were collected on eight reefs (Carysfort, Algae, Horseshoe, Grecian Rocks, French, White Banks, Molasses, and Conch) ranging in depth from 2 to 20 m. All sites were offshore of north Key Largo (ca. 25° 07'N, 80° 18'W), and included both patch and fringing reef environments.

Sampling was conducted during three field trips in February, June, and August 1994 with water temperature ranging from 24 °C (February) to 29°C (June and August). Samples were limited to visible populations of microalgae, located by searching for biofilms, tufts, and patches with characteristic red or bluegreen cyanobacterial pigmentation. Samples were photographed in situ using macro-photography, and collected using sterile 60 ml syringes or hand-collected and placed in sterile reclosable bags. All samples were examined microscopically within 7 h of return to shore. Subsamples were preserved with Lugol's solution, and cyanobacteria were identified according to Bergey's Manual of Systematic Bacteriology (1989). *P. corallyticum* was identified based on morphological characteristics (Fig. 1 bottom) which include cell width and length (4.4 and 4.0 μm respectively), occurrence of one pointed tip and one rounded tip, lack of distinct polysaccharide sheath, the presence of phycoerythrin, and active gliding motility. Samples of black band were also collected and examined.

Sites sampled which were found to contain free-living *P. corallyticum* filaments were monitored for periods of up to 17 mo to determine if black band disease developed.

Results and Discussion

Of 82 samples collected and examined, nine were found to contain *P. corallyticum*. Eight of these nine samples were collected from brown or red (i.e., phycoerythrin-rich) biofilms on the surface of sediment patches present in depressions on healthy scleractinian

corals of the species *Montastraea annularis* (species complex), *M. cavernosa*, and *Colpophyllia natans*. The sediment patches ranged from <1 to 2 cm wide by 1 to 4 cm long, and were covered by red, reddish brown, or dark brown, loose biofilms of microalgae (e.g., Fig. 2). In addition to *P. corallyticum*, other species of cyanobacteria were present. Species of *Oscillatoria* were found in every sample, while *Spirulina* was found in three of the eight samples, *Lyngbya* in two, and *Arthrospira* and *Phormidium* spp. in one. The sulfide-oxidizing bacterium *Beggiatoa* was present in four samples, and pennate diatoms in two.

One of the nine samples that contained *P. corallyticum* was from an isolated tuft on a denuded coral skeleton 14 cm from an active black band. This sample was the only one that contained solely *P. corallyticum* filaments. As the tuft was probably left behind by the migrating band, the number of samples with free-living *P. corallyticum* consisted of 8 out of 82 (10%).

The 74 samples that did not contain *P. corallyticum* consisted of epiphytic (n = 11), epizoic (n = 22), epilithic (n = 20), epipsammonic (n = 19) microalgal assemblages, and one microalgal tuft on metal. These samples were red, brown, orange, bluegreen, black, and purple in color. Epiphytic microalgae were collected from *Halimeda, Dictyota*, and *Asparagopsis*; epizoic samples were from dead and live gorgonians, the scleractinians *Diploria, Colpophyllia, Montastraea*, and *Favia*, and the hydrocoral *Millepora*; epilithic samples were collected from denuded areas on *Montastraea, Diploria*, and *Colpophyllia*; and epipsammonic samples were from both bottom sediments and sediment patches in depressions on corals. All of these samples consisted of mixtures of cyanobacteria or filamentous microscopic red algae. Cyanobacterial species were present of the genera *Spirulina, Oscillatoria, Lyngbya, Arthrospira, Phormidium, Schizothrix*, and *Anabaena*. Microscopic red algae (Rhodophyta) were not identified since classification of the members of the Rhodophyta requires identification of reproductive structures and post-fertilization events. (Many of the microscopic filamentous red algae morphologically resemble microscopic life-cycle phases of macroscopic species.)

Seven of the 22 epizoic samples were from dense red or maroon-colored microalgal populations on the surfaces of gorgonians. These consisted of either phycoerythrin-rich cyanobacteria or filamentous red algae. While gorgonians (species of *Gorgonia, Plexaura*, and *Pseudopterogorgia*) have been previously reported to be susceptible to black band disease (Antonius, 1985; Feingold, 1988), *P. corallyticum* was not present on any of these gorgonians sampled in this study.

A total of 23 microalgal samples were collected from healthy scleractinian corals (i.e., colonies with only healthy tissue), thus the eight samples containing *P. corallyticum* represented 35% of healthy corals investigated. Six additional scleractinian corals which did not appear healthy were also sampled. These corals had large denuded areas, or were blotched or mottled. Thus, a total of 29 scleractinian corals (not including those with black band disease) were sampled. The presence of *P. corallyticum* on eight of these corals constituted 26% of this subgroup.

Four of the 8 samples from uninfected corals which contained *P. corallyticum* also had filaments of *Beggiatoa* spp., a sulfide-oxidizing genus which lives exclusively in an O_2-sulfide interface (Jørgensen and Revsbech, 1983). Two of the eight samples contained diatoms, which are generally obligate aerobes that cannot tolerate conditions of anoxia or sulfide. Thus the distribution of free-living *P. corallyticum* included both aerobic and oxic/sulfidic interface environments. Six of the patches containing *P. corallyticum* on

healthy corals were monitored periodically for a period of 12-17 mo, from the time of discovery (February or June, 1994) through June 1995. Both aerobic and oxic-anoxic interface environments were represented. None of these patches exhibited symptoms of black band disease, although on the same reefs other coral colonies developed new or recurrent black band infections. None of the corals observed to develop black band disease were initially infected with white band disease, as reported to occur by Antonius (1981).

The identification of *P. corallyticum* in this study was based exclusively on filament morphology, pigmentation, and motility as determined using light microscopy, and as presented in the definitive species description of Rützler and Santavy (1983). The highly distinctive morphological characteristics, detected using light microscopy, have been the basis for field identifications of *P. corallyticum* in most studies reported. As there have been no genetic analyses carried out for taxonomic identification of this species, species verification using molecular probes could not be conducted. Similarly, *P. corallyticum* is not presently in any existing culture collection (although we will be sending our laboratory culture to the Pasteur Cyanobacterial Culture Collection in 1996). In addition, since the black band pathogen has not been identified using Koch's postulates, pathogenicity could not be utilized as a criterion for identification. Verification of the free-living *P. corallyticum* as the same strain as the black band *P. corallyticum* is, therefore, not currently possible, however the morphology-based identification is the first successful attempt to document this species not associated with black band.

Although the eight microalgal populations which contained *P. corallyticum* did not develop into active BBD, and the mechanism of BBD initiation under natural conditions remains a mystery, the frequency of the presumed pathogen on healthy corals is surprising. The occurrence of *P. corallyticum* on more than one third of healthy scleractinian corals, but in none of the other environments sampled (n = 59), suggests that the surface of healthy corals is a reservoir for this cyanobacterium. Continued effort should be made to determine the mechanism of disease initiation and transfer, and particular effort should be made to determine if stress or injury is involved. Previous reports (Antonius, 1981) that the incidence of black-band disease is positively correlated with nutrient increase and eutrophication also serve as an impetus for further research in this area.

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