

ECOLOGY OF THE BLACK BAND DISEASE MICROBIAL CONSORTIUM

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ABSTRACT

Black band disease of scleractinian corals consists of a suite of microorganisms that is directly analogous to microbial mat communities except that it migrates across, and destroys, living coral tissue. We have been characterizing the black band microbial consortium in terms of physiology and motility patterns. The black band microorganisms are finely adapted to each other in terms of generation and maintenance of, and physiological response to, dynamic microgradients of O_2 and sulfide. Microbial maintenance of anoxia and sulfide at the base of the consortium is toxic to coral tissue, and artificial exposure of coral to sulfide and anoxia in controlled incubations results in coral death similar to that caused by black band disease. Thus the microbial ecology of black band disease may relate to the overall ecology of the disease on the reef in that the microbially mediated chemical microenvironment of black band disease is responsible for coral mortality.

INTRODUCTION

Previous studies of black band disease of scleractinian corals (Antonius 1981; Rützler et al. 1983; Richardson, in press) have revealed that black band is always composed of a distinct microbial consortium. This finding is in contrast with the results of studies of other microbial diseases of corals, such as white band disease, which exhibit highly variable populations of microorganisms (Peters et al. 1983). Attempts to document recurrent microbial members within the microbiota of other coral diseases have been unsuccessful (Peters 1984).

The highly characteristic microbial population of black band is dominated by the phycoerythrin-rich, filamentous, gliding cyanobacterium *Phormidium corallyticum* (Rützler and Santavy 1983; Taylor 1983), also reported as present in black band infected octocorals (Antonius 1985; Feingold 1988). *Phormidium corallyticum* in black band infections of scleractinians is usually accompanied by populations of another filamentous, gliding bacterium, the (non-photosynthetic) sulfide-oxidizing genus *Beggiatoa* (Garrett and Ducklow 1975). Numerous heterotrophic bacteria (Ducklow and Mitchell 1979) and marine fungi (Ramos-Flores 1983) are also present in black band, as well as a population of sulfidogenic bacteria (Garrett and Ducklow 1975), recently confirmed to be *Desulfovibrio* spp. using 16S rRNA-targeted oligonucleotide probes (Schnell et al. 1996). While the above reports are all consistent in their descriptions of the microorganisms present in black band, none have focused on discerning the details of the microbial ecology of black band disease.

We have recently demonstrated, using microelectrodes, (Carlton and Richardson 1995) that the black band community is structurally and functionally similar to microbial mats found in sulfide-rich, illuminated aquatic environments. The dominant cyanobacterium, *Phormidium corallyticum*, carries out oxygenic photosynthesis during the day, which results in a subsurface maximum of (supersaturated) O_2 . Aerobic conditions extend several hundreds of μ m down into the 1mm thick band itself, but the base of the band is always anoxic. An oxic/anoxic interface migrates vertically within the band on a diel basis in response to change in photosynthetic activity. Dynamic microgradients of sulfide are also present within the

band. Throughout the day, sulfide is present only at the very base of the band. With the absence of photosynthetic O_2 evolution at night, however, sulfide accumulates to concentrations of $>800 \mu$ M, and is present all the way to the band surface (Carlton and Richardson 1995). As the microzone of overlapping oxygen and sulfide migrates vertically over time the population of sulfide-oxidizing *Beggiatoa* follows, while the *Phormidium corallyticum* population remains stationary (Richardson, in press).

The chemical microstructure of black band can be viewed as a dynamic, vertical zonation of microenvironments which range from a permanently anoxic and sulfide-rich environment (at the base of the band) to a fluctuating surface environment exhibiting supersaturation of O_2 during the day and aerobic conditions with low levels of sulfide at night. These environmental parameters have been shown to dramatically affect the physiology and motility patterns of microbial mat microorganisms (Jørgensen 1982; Jørgensen and Revsbech 1983; Møller et al. 1985; Cohen et al. 1986; Richardson and Castenholz 1987a,b). The black band microbial community is not only well-adapted to metabolically utilize these extreme conditions, the chemical microstructure is itself generated and controlled by the metabolic modes of the black band microorganisms themselves. The maintenance of anoxia and sulfide at the base of the band has previously been suggested to be the mechanism of coral death (Carlton and Richardson 1995).

This paper presents recent results of our studies, and integrates our previous findings on the relationships between the ecology of the microorganisms within black band and coral mortality.

MATERIALS AND METHODS

Microelectrode measurements of O_2 and H_2S
Details of the microelectrode technique are described by Carlton and Richardson (1995). In brief, measurements were made on intact black band present on cores of *Montastraea annularis*. The cores were collected using a pneumatic drill equipped with a 4 cm diameter hole saw and immediately transported to the laboratory. These cores were maintained in a 10 l flow-through aquarium. Both O_2 -sensitive and sulfide-sensitive microelectrodes (tip diameters 30-40 μ m and 50-60 μ m respectively) were inserted into the black band using separate micromanipulators which allowed vertical positioning of the electrode tips at a resolution of $<10 \mu$ m. The O_2 microelectrode was calibrated against air-exposed seawater (21% O_2) and anoxic seawater (sparged for 30 min with N_2). The sulfide microelectrode was calibrated against freshly prepared solutions of $Na_2S \cdot 9H_2O$ at concentrations of 0, 50, 100, 250, 500, and 1000 μ M. The microelectrode signals were measured using a Diamond General model 1201 picoammeter (for O_2), and a Diamond General model 1300 Electro-Chem Analyzer (for H_2S). The outputs of both meters were recorded on a Kipp and Zonen type BD112 dual pen chart recorder. In this report, total sulfide (i.e. $[H_2S] + [HS^-] + [S^{2-}]$) will be reported as $[H_2S]$, with final values corrected for pH.

Light measurements

During the O_2 and H_2S measurements, natural sunlight was adjusted using a neutral density screen to an intensity similar to that measured *in situ*. Light (photosynthetically active radiation, 400-700 nm) was measured using a Biospherical Instruments Inc. (model

QSL 100) averaging quantum meter with the sensor placed in the aquarium near the black band sample. Light readings were continuously output to a second Kipp and Zonen recorder. *In situ* light was measured using a diver-operated Biospherical Instruments Inc. (model QSI 140) scalar irradiance meter.

Toxicity studies

Toxicity studies were performed on 2 cm cores of *Montastrea annularis* collected as described above. Cores were incubated in 50 ml jars in a 35 l laboratory aquarium under the following conditions: light-oxic; dark-anoxic; and dark-anoxic with sulfide. Dark incubations were double-wrapped in black cloth, which did not allow light to penetrate as measured with the light meter. Anoxic and anoxic-sulfide incubations were wrapped as the base of black band is dark (as determined by microelectrode studies which revealed the lack of photosynthetic activity at depth in the band). Oxic incubations were exposed to the seawater in the aquarium (bubbled continually with air); anoxic incubations were sparged with N_2 for 30 min and capped with stoppers; and sulfide incubations were prepared by adding sulfide to anoxic seawater in jars before capping. Final sulfide concentration was 800 μM sulfide (added from a 1 M stock solution of $Na_2S \cdot 9H_2O$), the maximum concentration of sulfide we have measured at the base of intact black band (Carlton and Richardson 1995). Cores were examined for periods of up to 17 hours (the calculated average time it takes for a 2mm wide black band to pass over a fixed point on the surface of a coral colony). At each time point, controls (light-oxic) were examined under a Bausch and Lomb dissecting scope for general appearance, and touched gently with a pipette to determine polyp response. Dark-anoxic and anoxic-sulfide incubations were harvested after different incubation periods by removing the experimental cores from the incubation jar and examining them in the same manner as the controls.

RESULTS

Four representative microprofiles of O_2 and H_2S gradients within intact black band disease, during both daylight

(right panel) and darkness (left panel), are shown in Figure 1. These microprofiles are typical of those we have measured previously. The variability in O_2 and sulfide on a daylight vs. darkness basis is primarily due to the presence vs. absence of light-dependent oxygenic photo-synthesis. As such, while sulfide production in the dark lower zones of the band occurs both day and night, chemical oxidation by O_2 results in daytime H_2S depletion.

During daylight (right panel), the O_2 maximum was present at approximately 100 μm into the band, within the surface population of *Phormidium coralliticum*. Supersaturation of O_2 was observed within the upper 300 μm , and O_2 concentration decreased above the band surface, the result of diffusion into the water column. O_2 was absent below 500 μm . Sulfide was present at depths below 400 μm , and increased in concentration to $> 100 \mu M$ at 600 μm into the band. There was a slight zone of overlap of O_2 and sulfide between 400 and 500 μm . We routinely do not measure microprofiles all the way to the surface of the coral colony as the glass microelectrode tips will most certainly break upon contact with coral skeleton. Therefore, in these and other profiles, we have not measured the maximum sulfide concentration which is present at the base of the band.

During darkness (left panel), sulfide was present throughout the band to the surface, and was present in water immediately overlying the band. Sulfide concentration increased to a maximum of 560 μM at a depth of 600 μm . An anoxic condition was created below 200 μm , and O_2 was subsaturated between 200 μm and the band surface. There was a zone of overlap of O_2 and sulfide between the surface and $\sim 200 \mu m$.

While Figure 1 shows stable microprofiles at two time points during darkness vs. moderate light intensity, the microprofiles within the band were actually very dynamic in terms of both vertical positioning of the O_2 -sulfide interface and concentrations of O_2 and sulfide. Both values change as light intensity varies throughout the day, with the most extreme variability occurring on a day vs. night basis for each depth within the band. Table

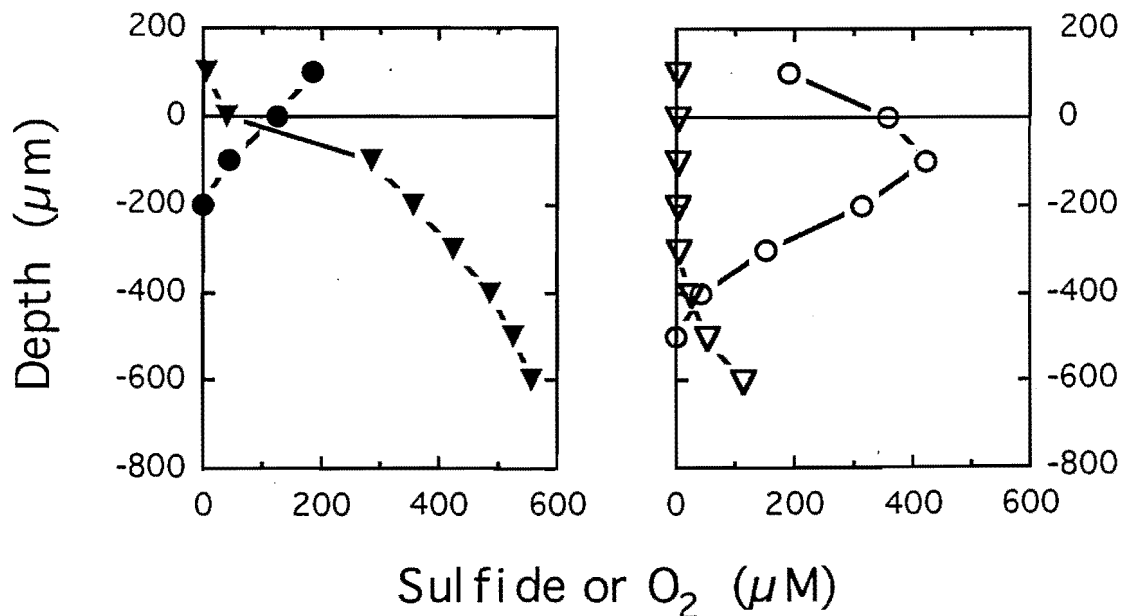


Fig. 1: Microprofiles of oxygen and sulfide in black band on *Montastrea annularis* during darkness (left panel) and daylight (right panel). Light intensity during the daylight recording was 478 $\mu mol\ quanta\ m^{-2}\ s^{-1}$. On the vertical axes, 0 is equivalent to the surface of the band with negative values corresponding to depth into the band. Round symbols represent O_2 concentration, triangles represent sulfide; closed symbols represent darkness, open symbols daylight. Air saturated O_2 equilibrium was 190 $\mu M\ O_2\ l^{-1}$.

1 shows the vertical migration of the depth of the O_2 -sulfide interface, and the ranges in $[H_2S]$ at two depths within the band, during day vs. night measurements. These values were calculated from a variety of measurements made in black band on cores of *Montastraea annularis* during 1993 and 1994. $[O_2]$ showed similar variations (data not shown).

Table 1: Range of values of microelectrode measurements made in black band on cores of *Montastraea annularis*. Microelectrode readings were taken at 100 μm intervals into the band. Each value is the mean of four measurements, with standard deviation shown in parentheses.

Parameter Measured	Day	Night
Depth of O_2 - H_2S interface	400 μm (0)	<100 μm (0)
H_2S concentration at 100 μm	0 μM (0)	312 μM (28)
H_2S concentration at 500 μm	44 μM (15)	443 μM (81)

Both Figure 1 and Table 1 show that the microenvironment at the bottom of the band, near the coral colony, is anoxic and sulfide-rich. It is possible that exposure to these conditions is the mechanism of coral death by black band since it is known that sulfide is toxic to eukaryotes (Vismann 1991). To test this hypothesis, we artificially exposed coral polyps to the same chemical conditions present at the base of black band. Results of the coral toxicity studies are summarized in Table 2. Exposure of cores of *Montastraea annularis* to conditions of anoxia and anoxia/800 μM sulfide resulted in tissue lysis, loss of zooxanthellae within remaining tissue, and exposure of coral skeleton. These responses were evident in the cores exposed to anoxia and sulfide after 12 h of incubation, which is the length of time it would take a 1.5 mm wide black band to pass a fixed point on a coral colony when migrating at the average rate of 3 mm d^{-1} (Richardson, in press). This width corresponds to the minimum we have observed in the field (personal observations). Dark-anoxic incubation also resulted in coral mortality. Coral polyps appeared extremely stressed after 12 hours of exposure, with tissue lysis and exposure of coral skeleton occurring after 17 h.

Table 2: Response of *Montastraea annularis* to exposure to anoxic and anoxic/sulfide-rich incubation conditions. Coral replicates were exposed for periods of up to 17 h, the time it would take a 2 mm wide *in situ* black band to pass a stationary point on the surface of a coral colony when migrating at the average rate of 3 mm d^{-1} .

Incubation condition	Polyp response after incubation time (hours)			
	0 h	3 h	12 h	17 h
light (control)	polyps open, respond to touch	polyps open, respond to touch	polyps open, slow response	polyps open, no response
dark-anoxic	"	polyps retracted, no response	loss of zooxanthellae, no response	skeleton exposed, mucous & bacteria
dark-anoxic, 800 μM H_2S	"	polyps white, respond to touch	tissue lysis, bleached, no response	skeleton exposed, mucous & bacteria

The 17 h dark-anoxic coral samples appeared similar to the anoxic/sulfide exposed cores incubated for 12 h. During incubations of 3 hours, we did not observe tissue lysis or skeletal exposure in either dark, anoxic-sulfide or dark-anoxic corals. The polyps of dark-anoxic cores appeared healthy in pigmentation, but were retracted and did not respond to tactile stimulation. Cores exposed to dark, anoxic-sulfide conditions for 3 h were also retracted and non-responsive, but appeared white. Coral polyps in the controls remained healthy in appearance, but exhibited an overall slowing of tactile response until they were unresponsive (17 h). This decline in response may be due depletion of food source

within the aquarium, even though the water was changed every 3 hours.

DISCUSSION

The black band disease microorganisms are physiologically finely adapted to the vertical zonation of O_2 and sulfide microenvironments present within the band. The population of the gliding genus *Beggiatoa* migrates along with the vertically moving O_2/H_2S zone of overlap, which allows this microorganism to maintain the preferred metabolic mode of sulfide-oxidation (Jørgensen 1982; Jørgensen and Revsbech 1983). The cyanobacterium *Phormidium corallyticum*, which does not vertically migrate but forms the matrix for the horizontally migrating consortium, is able to tolerate both O_2 supersaturation (generated by oxygenic photosynthesis of this species) and anoxia coupled with sulfide. *P. corallyticum* switches between oxygenic and anoxygenic photosynthesis using sulfide as electron donor (unpublished) depending on environmental sulfide concentration. Thus *P. corallyticum* physiologically adjusts to the dramatic chemical changes which occur in the subsurface zone. The population of *Desulfovibrio* spp. dominates, and remains at, the base of the band (W Goldberg, pers. commun.), a microenvironment which is always anoxic. This microenvironment is rich in organic carbon and nutrients (from lysing coral), which allows the development of populations of up to 2.2×10^8 cells per g fresh weight black band (assayed with lactate as substrate; Schnell et al. 1996).

The high levels of sulfide (up to 800 μM measured) generated by the *Desulfovibrio* population probably aids the black band microbial community by excluding microbial species intolerant to sulfide exposure (such as eukaryotic microalgae and obligately aerobic bacteria). Accumulated sulfide may have an additional benefit to the black band community in that it appears to repel grazing by fish; we have often observed fish taking a bite of black band and immediately spitting it out into the water column. Of more importance, however, is the role of sulfide in coral mortality by black band,

which apparently shortens the time period leading to death. Although dark-anoxic conditions result in coral tissue death and lysis, coral death occurs after a time period which is longer than the exposure of coral to the smallest sized migrating black bands (which effectively kill coral).

Based on the above, the black band microbial community can be construed to function together to generate and maintain an extreme microenvironment of anoxia and sulfide adjacent to living coral tissue. The lysing coral tissue, killed by prolonged exposure to this extreme chemical environment, provides a source of

organic carbon and nutrients to sustain the high rate of sulfidogenesis. The consortium migrates horizontally towards living coral tissue, allowing continual renewal of the source of organic carbon (i.e. fresh coral tissue). The individual members of the microbial community thus either tolerate, or make metabolic use of, the temporally and/or spatially fluctuating microenvironments of oxic vs. anoxic and sulfide-rich conditions which exist within the 1 mm thick band.

The microbial ecology of black band disease should provide clues as to the overall disease etiology, as well as the basis for regional differences in black band disease abundance and distribution (Antonius 1988; Edmunds 1991; Kuta and Richardson, in press). Many questions concerning black band disease, however, remain unanswered. Further research on all aspects of black band disease etiology should be encouraged, especially since black band disease is considered to be a factor in the perceived decline in coral reef health on a global basis (Williams and Bunkley-Williams 1990; Porter and Meier 1992).

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