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Cover Photo: Dr. Lynn Margulis, Symposium Keynote Speaker, describes the structure and ecology of living stromatolites. Some, visible as grayish mounds near her feet, line the shore of Storrs Lake whereas others occur farther out in deep water. (See paper by D. C. Edwards, this volume).

Back Cover Photo: Group photo of the 6th Symposium participants and speakers.

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INT-LINKED DEHYDROGENASE ACTIVITY IN THE SURFACE MUCOPOLYSACCHARIDE LAYERS OF BAHAMIAN SCLERACTINIAN CORALS

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ABSTRACT

Bacteria comprise part of the 'normal microbiota' of the surface mucopolysaccharide layers (SML) of hard corals. There is evidence that these organisms may nutritionally enrich the coral mucus, which may then be ingested by the coral animal. Other ecological roles of SML bacteria may include protection against pathogens and mineral transformations. Very little, however, is known about the identity or activities of these communities. SML samples were taken throughout 1993-94 from a variety of coral species growing off the coast of San Salvador, Bahamas, and used to estimate overall metabolic activity. We used a dehydrogenase assay based on the competitive inhibition of oxidized NAD by tetrazolium (INT) to bind with the enzyme. Rates of formazan production (INT-reduction) were higher for samples from bleached corals (*Acropora cervicornis* and *Porites asteroides*) than from nonbleached stands. In general, overall activity was higher for large boulder-type corals than for encrusting or branching species.

INTRODUCTION

Living corals produce a covering of mucoid material, much of which is lost to the surrounding seawater (Means and Sigleo, 1986). Particulate nutrients can be trapped in this material (Lewis and Price, 1977), and accumulated sediment can be removed by overproduction and elimination of mucus sheets (Hubbard and Pocock, 1972). This mucus layer is composed primarily of mucopolysaccharides (Daumus and Thomassin, 1977; Ducklow and Mitchell, 1979; Pascal and Vacelet, 1982). Mucolipids, rich in palmitoleic and myristic acids (Patton et al., 1977, Means and Sigleo, 1986), and amino acid hydrolysates, rich in glycine, glutamate, serine and alanine residues (Daumas et al., 1982; Ducklow and Mitchell, 1979; Means and Sigleo, 1986), make up a smaller porportion of the surface mucopolysaccharide layers (SML) of scleractinian corals.

The coral SML provides a habitat, as well as an oxidizable substrate, for resident heterotrophic bacteria (Ducklow and Mitchell, 1979; Rublee et al., 1980). Segel and Ducklow (1982) reported that elevated mucus production rates (due to pollution stress) also increased bacterial levels in the SML. Pascal and Vacelet (1982) suggested that the SML provides a matrix of framwork in which bacteria attach themselves. Bacteria have been shown to densely colonize this environment (Disalvo, 1971; Sieburth, 1975); they consume part of the mucus (Herndl and Velimirov,1986), and are themselves partly consumed by the coral animal (Sorokin, 1973). The SML may also be enriched through nitrogen fixation (Williams et al., 1987), thus increasing N:P ratios (Schiller and Herndl, 1989).

The composition of SML heterotrophic

bacterial communities has, until recently, received little attention. Bacterial community differences, based on carbon source utilization patterns between species and as a result of bleaching, were observed in *Montastrea annularis* and *Acropora cervicornis* (Ritchie et al.,1994a; 1994b; Ritchie and Smith, 1995a). Paul et al., (1986) reported increased levels of thymidine incorporation in the SML compared to surrounding seawater.

The purpose of this study was to compare overall metabolic activity in the SML from a variety of scleractinian coral species growing in oligotrophic waters of the Bahamas. Comparisons were also made between bleached and normal colonies when these were found. An INT-linked dehydrogenase assay was used to determine nonspecific overall activity.

MATERIAL AND METHODS

Sample Collection

Samples of the SML from *Montastrea annularis*, *Acropora cervicornis*, *A. palmata*, *Porites porites*, *P. astreoides*, *Agaricia agaricites*, *Dichocoenia stokesi* and *D. labyrinthiformis* were obtained from shallow patch reefs surrounding San Salvador Island, Bahamas using needleless 3.0 ml syringes. Seawater samples from reef sites, SML samples from isolated bleached colonies of *P. astreoides*, and SML samples from *Acropora cervicornis* showing symptoms of white-band disease were also taken. All samples were transferred to sterile vials on shore and placed in ice. These were kept at 3°C until laboratory assays were performed.

INT Assay

Samples were allowed to equilibrate at room temperature for three hours and vortexed at full speed for two minutes before 0.5 ml subsamples were added to reaction vials. Reaction vials contained 0.25 ml of a 1.5 mg ml^{-1}, 2-(p-Iodophenyl)-3-(p-nitrophenyl)-5-phenyl-2H-tetrazolium Chloride (INT, Kodak) and 0.5 ml of 2X GASW medium (Smith and Hayasaka, 1982). Reaction vials were then incubated in the dark at room temperature for three days. After three days vials were assayed for formazan activity, except for the *A. cervicornis* experiment which was sampled every 12 hours. The absorbance of reduced INT (Formazan) was measured spectrophotometricly at 490nm and concentration determined by comparison with a standard curve of triphenyl formazan (Kodak). Sterile artificial seawater treated identically was used as a control. Four replicates were run on all samples and controls.

RESULTS AND DISCUSSION

A time course showing the evolution of formazan from *A. cervicornis* samples is shown in Figure 1. The exponential increase in the

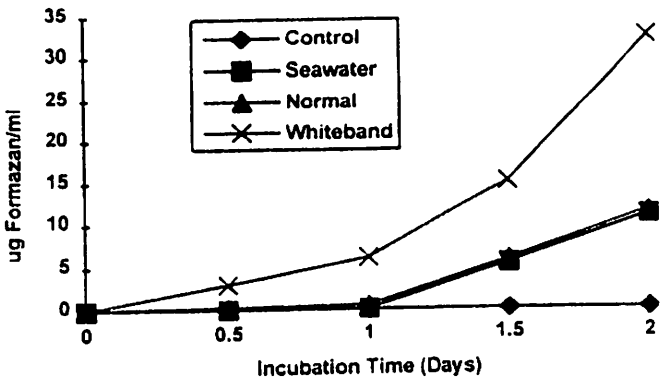

Figure 1. INT reduction of *A. cervicornis*

rate of formazan production with white-band samples indicates that the bacterial community was actively growing and metabolising. SML bacteria from normal corals were not much more active than seawater samples. Metabolic activity in seawater can vary considerably, depending on the amount of suspended sediment (Smith, 1988). Table 1 shows this variation in INT reduction from seawater samples obtained at different times. This variation was not as pronounced with coral SML samples and, in every case, INT reduction rates were higher for *A. cervicornis* samples showing white-band symptoms than for normal (uninfected) samples. White-band samples, then, appear to exhibit both a qualitative change in the composition as well as a greater level of metabolic activity of the SML bacterial community (Ritchie and Smith, 1995a). This might be due to the release metabolites resulting from the death of coral

Table 1. Temporal and spatial variation of INT reduction in the SML of *A. cervicornis*.

		μg Formazan ml^{-1}			
Site	Month	Control	Seawater	Normal	Whiteband
Potters Reef	6-93	0	21.5 (7.0)[a]	12.1 (4.8)	29.0 (4.3)
	8-93	0	0.2 (0.1)	10.5 (0.7)	22.3 (1.8)
Rocky Point	7-93	0	23.5 (8.7)	10.8 (1.3)	15.6 (1.0)
	10-93	0	10.2 (4.2)	22.9 (2.1)	26.2 (1.8)

Table 2. INT reduction of normal and bleached *P. astreoides* (Standard Error)

	μg Formazan ml^{-1}		
Control	Seawater	Normal	Bleached
0	0.02 (0.01)	0.18 (0.17)	5.59 (2.86)

Table 3. INT reduction in the SML of various scleractinian coral species.

Growth Form	Species	Sample Date	μg Formazan ml^{-1} (standard error)
Branching	*Acropora palmata*	7-94	12.8 (3.8)
		10-94	16.5 (4.4)
	Acropora cervicornis	7-94	30.0 (4.8)
		10-94	10.0 (2.3)
		2-95	23.6 (0.9)
	Porites porites	7-94	8.2 (0.6)
		2-95	28.0 (8.1)
Encrusting	*Porites asteroides*	7-94	14.5 (8.0)
		10-94	18.7 (6.6)
		2-95	16.7 (1.6)
	Agaricia agaricites	11-94	4.8 (2.2)
		2-95	23.5 (3.7)
Boulder	*Montastrea annularis*	3-94	25.1 (4.5)
		2-95	23.8 (2.0)
	Diploria labyrinthisformis	10-94	60.6 (18.8)
	Dichocoenia stokesi	2-95	22.4 (2.3)

tissue. Ritchie and Smith observed (1995b) that substrates, likely to be released by recent coral death, were preferential for white-band SML isolates. A 30-fold increase was also observed with SML samples from bleached *P. astreoides* over normal tissue (Table 2).

A comparison of INT reduction rates, in SML's of eight normal species of scleractinian corals sampled at various times in 1994, is presented in Table 3. Among the branching growth forms, both *A. cervicornis* and *P. porites* showed a three-fold temporal variation. *Agaricia agaricites* had the lowest rate of INT reduction (11/94), as well as the greatest temporal variation (five-fold difference). The highest INT reduction rate was measured from *D. labyrinthiformis* samples. These samples were taken from a large colony, apparently recovering from sediment stress, so mucus production would be expected to be relatively high. Encrusting growth forms, in general, had the lowest rates of INT reduction (ave. 15.6 μg formazan produced ml^{-1}), followed by branching forms (ave. 18.4 (μg formazan produced ml^{-1}), and highest rates were observed with large boulder colonies (ave. 33.0 μg formazan produced ml^{-1}).

Although bacterial metabolic activity in the SML of sceractinian corals has been measured before (for example, Pascal and Vacelet, 1982; Paul *et al*, 1986), it has not, to our knowledge, been measured using a technique as nonselective as INT reduction (Pamatmat, 1977). This assay has been sucessfully used in other marine environments (Smith and Hayasaka, 1986) and, since dehydrogenase catalysed reactions are essential to all heterotrophs, INT reduction can be used as a general metabolic index.

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