BACTERIAL ECOLOGY OF SELECTED CORALS FOLLOWING THE 1994 SOUTH CENTRAL PACIFIC BLEACHING EVENT

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ABSTRACT

Syringe samples were taken from the surface of normal, bleached and necrotic corals from the South Central Pacific following the mass bleaching event which first appeared in March, 1994. Carbon source utilization patterns were determined for bacterial isolates from these samples and entered into a database from which community comparisons were made. Plate counts and INT-linked dehydrogenase measurements were performed on the samples to determine overall metabolic activity. Shifts in bacterial communities, based on cluster analysis, were observed with *Acropora* and *Pocillopora* species. Bleached communities clustered together, indicating a common shift in bacterial groups. In addition, an increase of 30 to 40 percent in INT-linked dehydrogenase activity was observed in bleached over normal samples in these two genera. Data observed for *Porites* was not consistent with the other genera, although fewer samples of this genus were available.

INTRODUCTION

Coral bleaching can lead to the mass mortality of entire reef systems and has been observed in all of the world's oceans (Goreau and Hayes, 1994; Hayes and Goreau, 1991; Williams and Bunkley-Williams, 1990; Williams *et al.*, 1987). Pacific reefs appear to suffer relatively frequent mass bleaching events (Goreau and Hayes, 1994; Jokiel and Coles, 1977; 1990; Salvat,1992). The occurrence of mass bleaching has been correlated to increased sea surface temperatures (Glynn, 1991; Goreau *et al.*; 1993), but the effect of this phenomena on the ecology of organisms associated with the coral animal is very poorly understood.

The surface of living corals is covered by a mucoid material (Means and Sigleo, 1986) which appears to increase during stress (Segel and Ducklow; 1982). This surface muco-polysaccharide layer (SML) provides a matrix for bacterial colonization (Pascal and Vacelet, 1982), allowing the establishment of a 'normal microbiota' which may be characteristic of a particular coral species or indicative of health status (Ritchie *et al.*, in press). Both bacterial populations and activity were shown to be higher in the SML than in the surrounding water mass (Disalvo, 1971; Ducklow and Mitchell, 1979; Paul *et al.*, 1986; Rublee *et al.*, 1980; Sieburth,1975). Bacteria were shown to be consumers of the SML mucus (Herndl and Velimirov, 1986), as well as enriching this material through nitrogen fixation (Williams *et al.*, 1987; Schiller and Herndl, 1989), and, in turn, become food for the coral animal (Sorokin, 1973).

The structure of the heterotrophic bacterial community has, until recently, received little attention. Ritchie *et al.* (1994a) reported a shift from *Pseudomonas* sp. to *Vibrio* sp. when *Montastrea annularis* became bleached. Ritchie *et al.* (1994b) also recovered isolates resembling *Vibrio charcharia* from *Acropora cervicornis* showing symptoms of white-band disease, but not from healthy tissue. A comparison of the bacterial communities in the SMLs of the two coral species showed differences in the distribution of metabolic groups and also differences when coral tissue became bleached (Ritchie and Smith, 1995). Le Campion-Alsumard *et al.* (1995a; 1995b) have also suggested a normal endolithic biota consisting of cyanobacteria, fungi and algae inside skeletal layers of *Porites lobata*.

Here, we report results of studies on the SMLs from normal, bleached and necrotic coral tissue obtained from three genera of hermatypic corals in the South Central Pacific. Samples, obtained after mass bleaching began in March 1994, were analyzed for heterotropic bacterial population levels, metabolic activity and community structure.

MATERIALS AND METHODS

Sampling and Strain Isolation. SML samples were taken from corals near three South Central Pacific islands from August 7 through 18, 1994, following a mass bleaching event which began in March of the same year. Bleached and unbleached samples of *Porites lobata* and *Pocillopora verrucosa* were obtained from inside fringing reefs on the north side of Moorea, French Polynesia, in water ranging in depth from three to five M.Water samples, near the coral, were also taken. Bleached, unbleached and necrotic areas of *Acropora* sp., as well as, bleached and unbleached areas of *P. verrucosa* were sampled in 20 M of water at the outer slope of Black Rock, on the west side of Rarotonga, Cook Islands. Water mass samples, near corals, were also taken. Bleached, unbleached and necrotic *P. verrucosa* samples were obtained from the north coast outer slope of Tutuila Island, American Samoa at 10 M depth. Bleached *Acropora* sp. and bleached, unbleached and necrotic *P. verrucosa* were sampled from the south side outer slope of this island, along with water samples at 20 M depth. All samples were taken using 3.0 ml syringes and contents were transfered to sample vials, insulated and transported to the laboratory.

Microscopic examination of samples revealed bacteria embedded in mucilaginous material. Subsamples of each vial (0.1 and 0.01 ml) were removed and spread plated onto a glycerol artificial seawater (GASW) medium (Smith and Hayasaka, 1982). Plates were incubated at 30° C for three days. All detectable colonies arising on each plate, with a distinctive morphology, were restreaked until pure cultures were obtained. The number of different colonies obtained from each source are given in Table 1.

Table 1. Number of morphologically different colonies obtained from each sample source.

Site Depth Water	*Porites*			*Acropora*			*Pocillipora*		
	unbleached	bleached	unbleached	bleached	necrotic	unbleached	bleached	necrotic	
Moorea 5 M	3	3	5	---	---	---	3	4	---
Tutuila 10 M	---	---	---	---	---	---	3	11	21
Tutuila 20 M	7	---	---	---	8	---	9	9	10
Rarotonga 20M	6	---	---	15	4	8	7	4	---

INT Assay. Subsamples from each vial (0.5 ml) were allowed to equilibrate at room temperature for three hours after which they were added to reaction vials containing 0.25 ml of a 1.5 mg ml^{-1} solution of 2-(p-Iodophenyl)-3-(p-nitrophenyl)-5-phenyl-2H-tetrazolium Chloride (INT, Kodak) and 0.5 ml of 2X GASW medium. Reaction vials were then incubated in the dark at room temperature for three days. The absorbance of reduced INT (Formazan) was measured spectrophotometricly at 490 nm and concentration determined by comparison with a standard curve of triphenyl formazan (Kodak). Sterile artificial seawater was treated identically and used as a control. Four replications were run on all samples and controls.

Metabolic Testing and Grouping of Isolates. Bacterial isolates were streaked on GASW medium until pure cultures were obtained. Samples of pure cultures were removed from plates by resuspending in 2.0 ml of sterile artificial seawater (ASW). The suspension was transferred to test tubes containing 20.0 ml sterile ASW and the density adjusted so that absorbance fell between 0.130 and 0.143 (590 nm). The suspensions were then distributed into Biolog™ GN microwell plates (Biolog Inc., Heyward, CA). Each plate contained 96 microwells with a different carbon source (except one control microwell) and tetrazolium violet as an indicator of metabolic activity. Results were read on an automated plate reader and absorbance readings 40 percent higher than control wells were scored as positive (Bochner, 1989). In this way, carbon source utilization patterns (CSUPs) were determined for each isolate. Each CSUP was entered into a database as a 32 digit octal code and subjected to cluster analysis. Clusters were assigned taxons based on the similarity of isolates to each other and known strains. Cluster analysis of the distribution of taxons among bacterial communities was based on relative Euclidean distance.

RESULTS AND DISCUSSION

Bacterial counts were similar among all of the samples (Table 1). Although population levels appeared similar, overall metabolic activity varied considerably. Bacterial dehydrogenase activity was higher with bleached samples than with either unbleached or necrotic *Acropora* or *Pocillipora* samples.This was not so with *Porites* samples, but we may not have had enough samples of this species to make a reasonable generalization

(Table 1). With the exception of *Porites*, bleached samples had activity levels similar to the water mass samples. This is in contrast to the findings of Paul *et al.* (1986) and Rublee *et al.* (1980), but Ritchie *et al.* (in press) observed that dehydrogenase activity in the water mass varied over 10-fold depending on weather conditions and, presumably, the amount of suspended sediment.

Table 2. Plate counts and INT-linked dehydrogenase activity from the SML of Pacific corals.

Sample	Plate Count cfu ml^{-1} (X 10^4)	Formazan Produced $\mu g\ ml^{-1}$
Water Mass	1.6	34.9
Porites Unbleached	1.2	76.2
Bleached	1.0	12.9
Acropora Unbleached	1.6	28.2
Bleached	5.5	37.6
Necrotic	0.1	26.9
Pocillipora Unbleached	13.4	24.1
Bleached	8.7	34.6
Necrotic	2.2	23.7

Dendrograms generated from carbon source utilization patterns (CSUPs) of each of the isolates, resulted in the establishment of 14 taxonomic groups. These taxons (groups) are represented by known strains corresponding to the closest similarity index in Table 3. It should be noted that these taxons are CSUP groups and may or may not correspond to a grouping based on genetic taxonomy, such as rRNA sequence comparisons. Groups III and IV are expected to be very diverse since the CSUPs contained few positives. Many of the strains in these two groups were Gram positives and a number were Actinomycetes.

Table 3. Grouping of bacterial isolates from Pacific corals based on CSUP similarities.

Group	Metabolic Type
I	*Pseudomonas I (cichorii)*
II	*Deleya*
III	*Brucella*
IV	Unknown
V	*Alteromonas I (haloplanktis)*
VI	*Agrobacterium/Klebsiella*
VII	*Vibrio*
VIII	*Pseudomonas II (paucimobilis)*
IX	*Cytophaga*
X	*Alteromonas II / Agrobacterium*
XI	*Enterobacter*
XII	*Klebsiella*
XIII	*Pseudomonas III (putida)*
XIV	*Erwinia/Gilardi*

A comparison of the bacterial communities from the various sources was made by looking at the percentage contribution of each group to the overall community (Table 4). Group I isolates were only found in the bleached *Pocillipora* bacterial community, where they made up seven percent of the population. Group VI was found in both unbleached and bleached *Pocillipora*, but only in this genus. This *Agrobacterium*-like bacteria may be characteristic of the *Pocillipora* SMLs. Although *Klebsiella*-like bacteria (Group XII) were also found in the water mass, they were only found in bleached SMLs, never in unbleached. This community shift may be typical for stressed Pacific corals. The Group XIV isolates (*Erwinia*-like) were only found in necrotic samples.

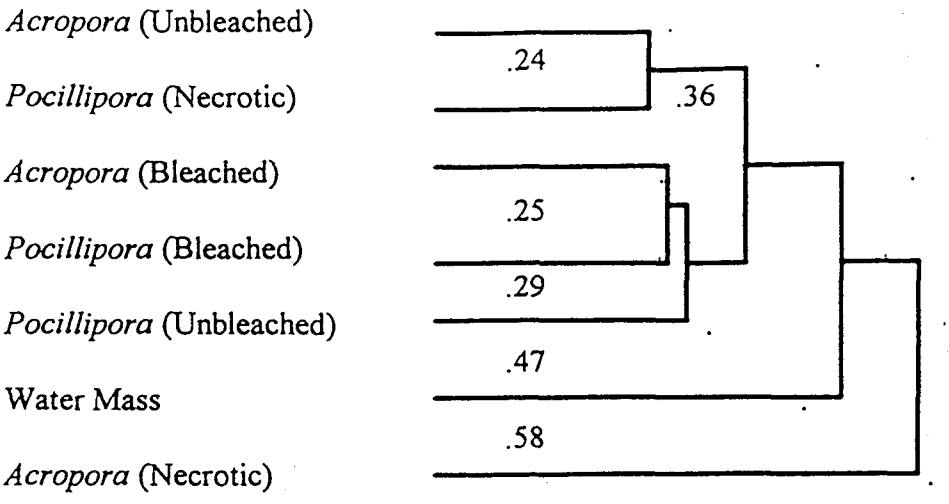
Table 4. Percentage of taxonomic groups isolated from Pacific coral samples.

		Porites		*Acropora*			*Pocillipora*		
Group	Water Mass	U*	B**	U	B	N***	U	B	N
I	---	---	---	---	---	---	---	7	---
II	---	33	---	14	17	25	9	3	19
III	12	---	40	29	8	12	9	3	19
IV	---	---	---	7	17	38	14	14	3
V	12	---	---	21	17	12	9	28	6
VI	---	---	---	---	---	---	18	3	---
VII	25	67	---	---	8	---	9	3	3
VIII	6	---	---	---	8	---	5	7	10
IX	---	---	---	7	---	---	---	---	6
X	12	---	---	---	8	---	5	3	6
XI	6	---	40	21	8	---	23	21	16
XII	19	---	28	---	8	---	---	7	3
XIII	6	---	---	---	---	---	---	---	3
XIV	---	---	---	---	---	12	---	---	3
Σ	98	100	108	99	99	99	101	99	97

*Unbleached **Bleached ***Necrotic

Based on the distribution of group taxa among bacterial communities, a dendrogram was constructed to show sample relatedness (Figure 1). *Porites* communities were not included because there were to few samples. Nevertheless, bleached *Acropora* and *Pocillipora* bacterial communities clustered together. Unbleached SML communities were not close and necrotic samples were not at all close to their living counterparts.

Figure 1. Dendrogram of bacterial SML communities based on relative Euclidean distance.



In summary, heterotrophic bacterial communities in the SML on normal, unbleached, scleractinian corals of different genera appear to be similar in population size and overall metabolic activity, but appear to differ in community composition. However, when bleaching takes place these communities become more similar in their distribution of taxons as determined by CSUPs. These results are based on only two genera, however; *Acropora* and *Pocillipora*. SML bacterial communities may be more similar among closely related coral species or species growing in similar geographic locations. We are currently increasing our CSUP database on a number of species from various locations in an attempt to determine the answers to some of these questions.

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