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BACTERIA ASSOCIATED WITH BLEACHED AND NONBLEACHED AREAS OF *MONTASTREA ANNULARIS*

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ABSTRACT

Reports of bleaching in corals and other organisms have been increasing in frequency over the past several years. These reports have stimulated a great deal of interest in the cause of this phenomenon, but little experimental work has been performed. This study began a systematic survey of bacteria associated with the surface of bleached and nonbleached areas of the scleractinian coral *Montastrea annularis*. In February 1993, a *M. annularis* was located in French Bay which showed areas of bleaching. Syringe samples were taken from bleached and nonbleached areas, including surface mucus layers and polyp tissue. Samples were transferred to vials, kept on ice, and plated out on a nonselective medium. Individual bacterial isolates were then restreaked, checked for purity and inoculated onto microplates containing 95 different potential carbon sources and a water control (BIOLOG PLATES TM). The oxidation of carbon sources produced a pattern characteristic of each isolate. These patterns were entered into a database from which comparisons of isolates from bleached and healthy areas were made. Isolate patterns were then grouped using cluster analysis so that potential pathogens could be indicated. Thirty percent of the isolates from bleached areas fell into a cluster corresponding to *Vibrio/Aeromonas* while none of the isolates from healthy areas fell into this cluster. This approach may allow the determination of pathogens involved in the cause or development of bleaching events.

INTRODUCTION

Coral reef ecosystems are among the most diverse in the world's oceans. However, increasing reports of coral bleaching indicate that these ecosystems may be in danger of deterioration over time (Glynn, 1993). A number of causes have been suggested for bleaching including elevated temperatures (Cook *et al.*, 1990) changes in salinity (Reimer, 1971), increased irradiance (Lesser *et al.*, 1990), various possible pathogens (Raghukumar and Raghukumar, 1991; Edmunds, 1991; Peter, 1984) and a normal process by which genetic variation among the zooxanthellae are maintained (Buddemeier and Fautin, 1993). Many other factors can also induce bleaching, but the significance of each factor is poorly understood. The mechanisms by which bleaching proceeds are also poorly understood.

Peters *et al.* (1983) and Peters (1984) demonstrated the presence of bacterial microcolonies in acroporid corals exhibiting white band disease, but the identity of the bacterium was unknown. Although bacteria are known to be abundant and active around corals and in the coral surface microlayer (Sorokin, 1973; Hobbie *et al.* 1977; Fuhrman and Azam, 1980; 1982; Paul, 1982; Segal and Ducklow, 1982; Paul *et al.* 1986), very little information exists on the structure or composition of this community. The purpose of this study was to determine the presence of

various metabolic types of bacteria associated with healthy surfaces of *Montastrea annularis* and compare them to metabolic types isolated from the surface of bleached areas.

MATERIALS AND METHODS

Study Site

A stand of *Montastrea annularis* with bleached areas was located in French Bay, San Salvador Island, Bahamas (Fig. 1). Bacterial samples were removed from the surface using a 3.0 cc syringe. Triplicate sterile syringes were used to obtain samples from both healthy and bleached areas. Contents of the syringes were transferred to sterile 1.5 ml vials on shore and kept on ice or in a 2°C cold room until plating in the laboratory.

Strain Isolation and Testing

Subsamples of 0.1 and 0.01 ml were spread plated onto a glycerol artificial seawater 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 transferred to new plates and checked for purity after three days. Colonies containing pure cultures were suspended in sterile artificial seawater at a density of between 0.130 and 0.143 (absorbance at 600 nm). The suspension was then distributed into Biolog TM plates. Each plate had 96 microwells containing 95 different carbon sources and a control. Each microwell contained tetrazolium violet to indicate metabolic activity with each carbon source. Results were read on an automated plate reader, and added to a database.

Data Analysis

Carbon source utilization patters (CSUP's) were entered into a data base as a 32 digit code (bionumber). Dendrogram construction involved clustering of heirarchical trees using a nearest neighbor unweighted pair group averaging cluster analysis technique. An individual cluster included all isolates showing less than 15 differences in CSUP's. Although isolates were not grown on the same medium as those in the GN database, coral isolate bionumbers were also compared to this database to determine taxonomic groups in general.

RESULTS AND DISCUSSION

Cell counts were higher from bleached areas (29.7 cfu/0.1 ml, S.E.= 9.7) than from nonbleached areas (13.7 cfu/0.1 ml, S.E.= 9.2), although differences were not statistically significant. The number of different colony morphologies per plate were significant (6.9, S.E.= 1.5 for bleached and 2.7, S.E.= 0.4 for nonbleached). This indicated a greater diversity of culturable heterotrophic bacteria from bleached areas compared to nonbleached areas. Bleached areas were also found to have less surface mucopolysaccaride layer than nonbleached areas. The absence of zooxanthellae may have affected the bacterial population. Zooxanthellae are known to increase surface mucopolysaccaride production which may suppress bacterial growth of certain strains or may provide a favorable carbon source for specific bacterial types. Either mechanism would decrease the population diversity.

The combined dendrogram (Fig. 2) revealed 13 clusters, A - M. Ten of these clusters contained five or fewer strains. Cluster H contained 30 isolates with approximately equal number from both bleached and nonbleached samples. Cluster I contained only 7 isolates, also split about half between bleached and nonbleached. These isolates probably represent members of the indigenous microflora for this coral species. Comparing this database with the Biolog database indicates that these bacteria (from Clusters H and I) fall within three large physiological groups. One group (subcluster H1), includes the genera *Deleya* and *Pseudomonas*, while subcluster H2 includes *Brucella* and others. This latter group, however, used very few of the carbon sources and may be relatively heterogeneous. All isolates from Cluster I corresponded to the genus *Alteromonas*. Both *Deleya nauticus* and *Alteromonas haloplanktis* clustered very closely to the coral isolates. Figure 3 shows the

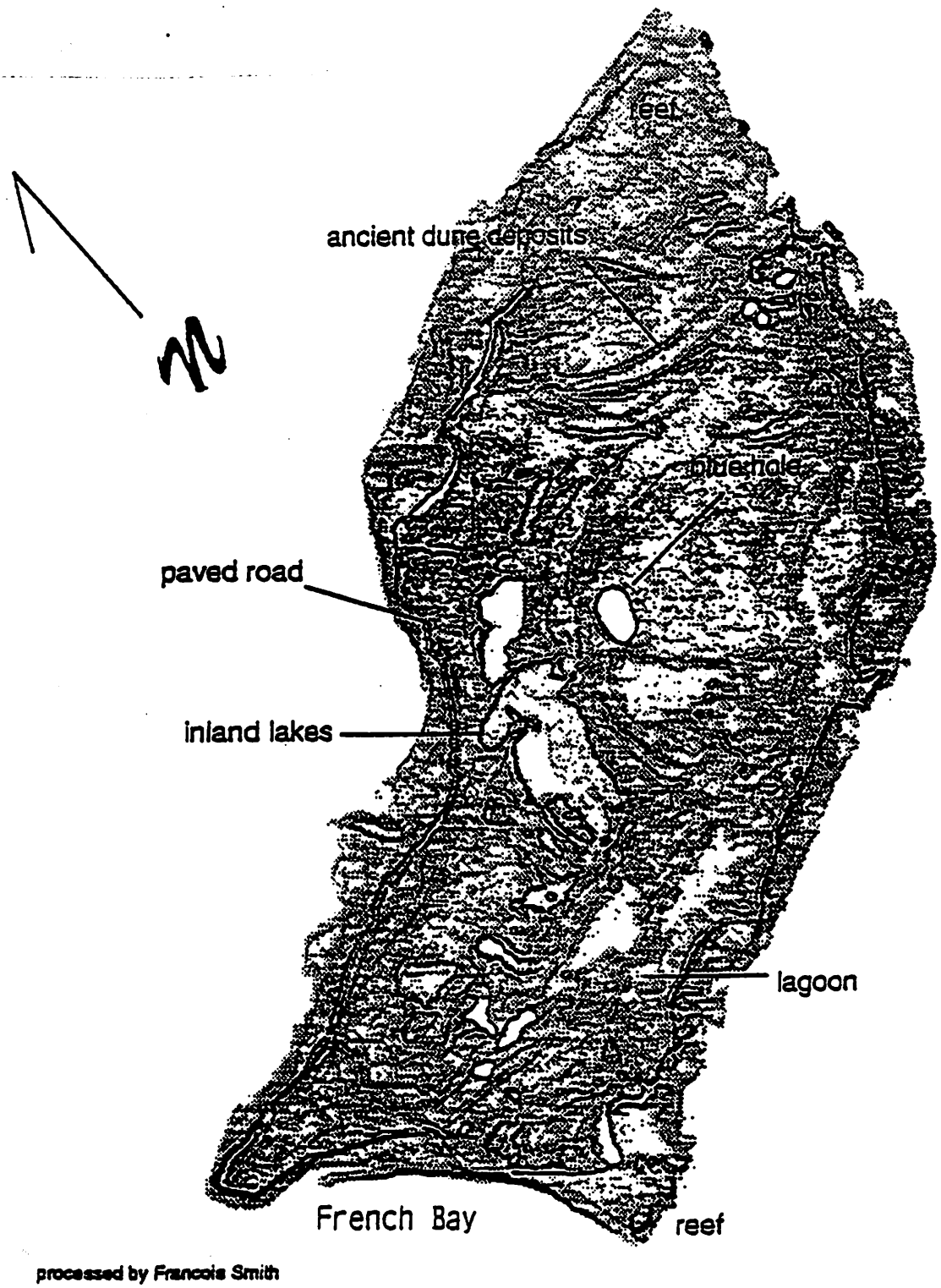

Figure 1. Edge Enhanced Landsat TM image of San Salvador, Bahamas, 1985 data.

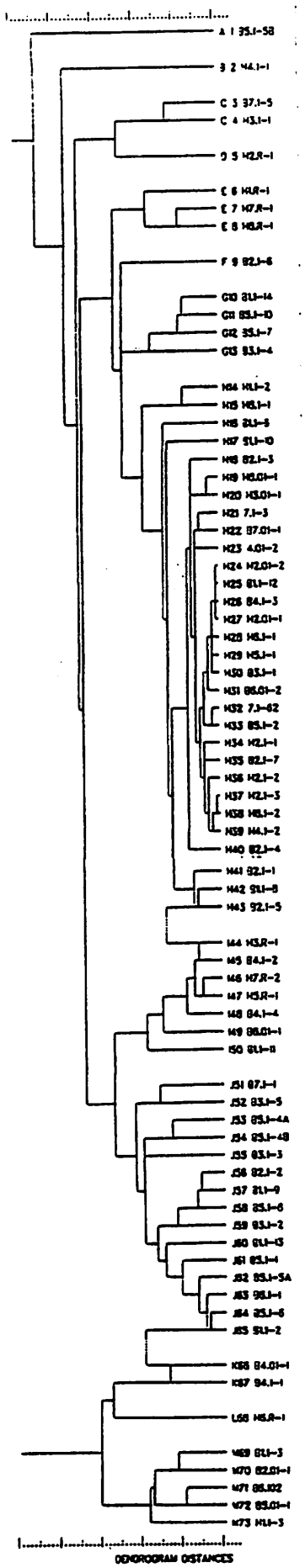

Figure 2. Combined dendrogram of bacterial isolates from bleached and nonbleached *M. annularis*. Notice that cluster J contains only isolates from bleached areas.

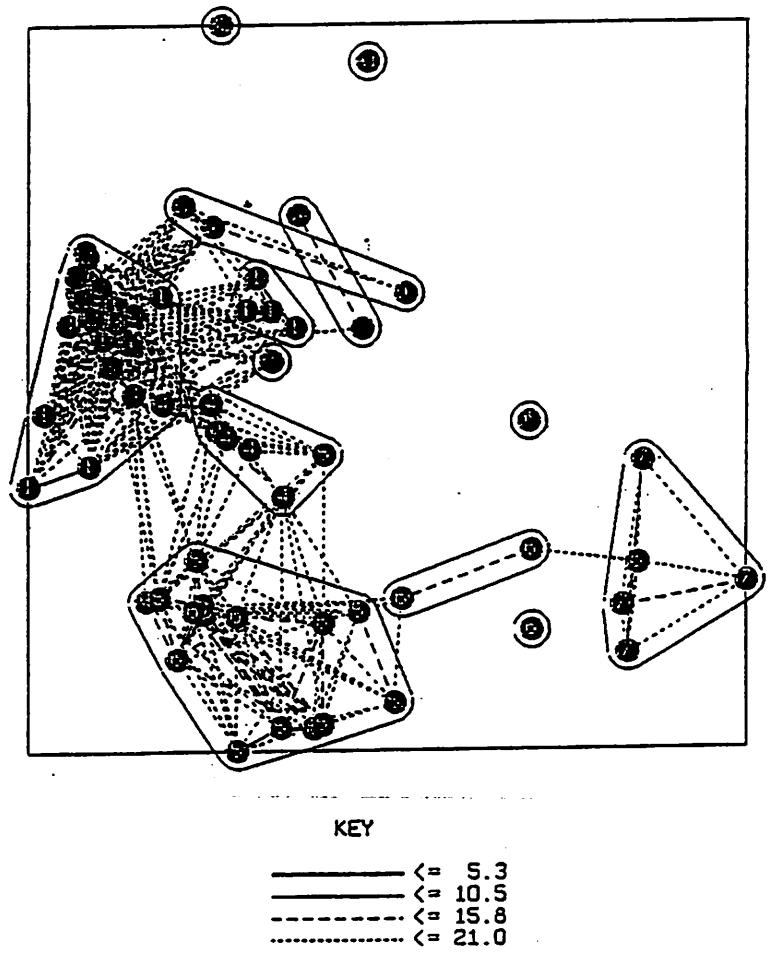

Figure 3. Two dimensional representation of the isolates contained in the dendrogram.

Table 1. Distribution of Bacterial Isolates among Taxonomic Groups from Bleached and Nonbleached *Monastrea annularis*.

Cluster	Group	%Nonbleached	%Bleached
A	*Acinetobacter/Alcaligines*	0	2
B	*Acidovorax/Pseudomonas*	4	0
C	*Sphingobacterium/Xanthomonas*	4	2
D	*Pseudomonas/Vibrio*	4	0
E	*Pseudomonas/Xanthomonas*	13	0
F	No Identification	0	2
G	*Deleya/Xanthomonas*	0	8
H1	*Deleya/Pseudomonas*	8	2
H2	*Brucella/Kingsella*	44	32
I	*Alteromonas/Vibrio*	12	8
J	*Vibrio/Aeromonas*	0	30
K	J and L	0	4
L	*Agrobacterium*	4	0
M	*Enterobacter/Klebsiella*	4	8

grouping of all clusters in two dimensions.

Thirteen percent of the nonbleached isolates fell into Cluster E (Table 1). No isolates from bleached areas were found in this Cluster. Thirty percent of all bleached isolates fell into Cluster J, with no representatives from nonbleached areas. This cluster strongly corresponded to the genus *Vibrio*. One isolate was identified as *Vibrio pelagius*. Although species identification must be suspect due to media inconsistency, Gram stains, cell and colony morphologies supported the genus identification. In addition, known strains of marine vibrios, grown on GASW fell into Cluster J. Because none of the bleached isolates were found associated with nonbleached areas, the occurrence of this group may be specifically associated with the bleaching event. Members of the genus Vibrio have been shown to be pathogens of a number of marine animals (Baumann *et al.*, 1984) and our results indicate that this may also be the case for corals.

SUMMARY

Results from this study indicate that the normal culturable heterotrophic bacterial community associated with *M. annularis* includes the genera *Deleya* and *Alteromonas*. Bleaching events appear to decrease the *Pseudomonas* population which is replaced by the genus *Vibrio*. Studies designed to determine the possible role of *Vibrio* sp. in the development of coral bleaching are continuing.

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