

PHYSIOLOGICAL COMPARISON OF BACTERIAL COMMUNITIES FROM VARIOUS SPECIES OF SCLERACTINIAN CORALS

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ABSTRACT

Bacterial communities within the surface layers of hard corals have been shown to play an important nutritional role for the coral animal. They may play an equally important role in maintaining the health and preventing disease in corals. However, very little is known about the composition of the 'normal microbiota' in the surface mucopolysaccharide layers of these animals. We attempted to address this problem by comparing carbon source utilization patterns of the bacterial communities from surface mucopolysaccharide layers. Each community was separately exposed to 95 potential carbon sources. Oxidation rates were measured every six hours for a 72 hour period. Most amino acids were oxidized by all bacterial communities. Oxidation of some carbohydrates and organic acids were highly variable. These were used to compare communities from the different sources. Cluster analysis showed that communities from the boulder corals grouped together (*Montastrea* spp., *Diploria* spp., and *Dichocoenia* sp.) and *Acropora palmata* clustered with *Porites* spp.. The *Agaricia agaricites* bacterial community was not similar to those of other corals. Finally, the bacterial community from the water mass was not similar to coral bacterial communities as a whole. These results indicate that corals may have a distinct microbiota characteristic of a coral type. Continuing studies should help maximize the level of resolution of these communities within specific coral species.

INTRODUCTION

The surface of living corals are covered by surface mucopolysaccharide layers (SMLs), the primary purpose of which appears to be the removal of particulate matter and perhaps protection against potential harmful organisms (Hubbard and Pocock, 1972; Means and Sigleo, 1986). Chemical analysis of SMLs from various corals have shown these structures to be rich in lipids, carbohydrates and protein (Benson and Muscatine 1974; Daumas and Thomassin 1977; Daumas et al. 1982; Patton et al. 1977; Ducklow and Mitchell 1979a; Pascal and Vacelet 1982; Coffroth 1990). Meikle et al. (1988) found that the glycose composition of mucus from different coral species varied considerably. The rate of mucus production was shown to increase when coral animals were exposed to stress (Ducklow and Mitchell 1979b; Segel and Ducklow 1982).

The coral SML harbors communities of heterotrophic bacteria (Sorokin 1973) by providing oxidizable substrates (Pascal and Valcelet 1981; Herndl and Velimirov 1986). It is likely that bacterial population levels are held in check by bacteriophages (Disalvo 1971). The nutritional interactions in the SMLs of different coral species would predictably lead to the establishment of a normal microbiota characteristic of that species (Ritchie et al. 1996a).

Corals stressed by disease or bleaching increased their production of mucus in the SMLs (Segal and Ducklow 1982). Concomitantly, SML bacterial communities also increase in number (Segal and Ducklow 1982) and activity (Ritchie et al. 1996b) when the corals are stressed. In addition, the composition of the SML bacterial communities also change (Ritchie and Smith 1995a; 1995b; Ritchie et al. 1994a; 1994b) due to stress. The bacterial communities did appear very closely attuned to their hosts metabolism (Ducklow and Mitchell 1979b).

The purpose of this study was to compare metabolic rates

of carbon source oxidations among SML bacterial communities from different scleractinian coral species. These communities could then be further characterized and comparisons among coral species evaluated.

MATERIALS AND METHODS

SMLs, containing the microbial communities, were collected from shallow (less than 5m) patch reefs in San Salvador, Bahamas (24°1'N by 74°10'W) using 30.0-ml needle less sterile syringes. Triplicate samples of the following coral species were obtained: *Montastrea annularis*; *M. cavernosa*; *Diploria labyrinthiformis*; *D. strigosa*; *D. clivosa*; *Porites divaricata*; *P. furcata*; *Dichocoenia stokesi*; *Agaricia agaricites*; *Acropora cervicornis* and *A. palmata*. Water mass samples surrounding the corals were also taken for comparison. All samples were kept on ice or in a coldroom (4°C) until metabolic testing in the laboratory.

Syringes were allowed to equilibrate at room temperature for three hours after which subsample dilutions were spread plated on a nonspecific medium (Smith and Hayasaka 1982). Samples were dispensed into Biolog GN microtiter plates (Bochner 1989) which contain 95 different potential carbon sources, each in a separate microwell, and a water control well. All microwells contained salts and a tetrazolium dye to indicate oxidative activity. Plates were read every six hours for 72 hours on an automated plate reader.

Wells showing a 40% increase in absorption at 490nm over controls were scored as positive at the end of the 72h incubation period and used to determine bionumbers. The bionumbers were used as a data reduction technique for presentation. This reduced the 95 test results to a 16 digit number which was the sum of values assigned to sequential microwells in groups of three that were positive. Positive values were 1, 2 and 4, respectively, for each of the three wells (containing different carbon sources). Bionumbers were then evaluated and the more variable carbon sources among the communities were compared. All tests were run in triplicate. There was negligible variation for any particular coral species-carbon source combination.

RESULTS AND DISCUSSION

Bacterial communities from the SMLs removed from *Diploria strigosa* oxidized a greater number of carbon sources than communities from any other source (83 of the 95). This was followed by communities from *M. annularis* (78), then *D. labyrinthiformis* (73). In general, the boulder coral (*Montastrea*, *Diploria* and *Dichocoenia*) bacterial communities used a greater variety of carbon sources (mean 70.2, std. dev. 9.8) than did communities from other coral species (mean 52.4, std. dev. 13.4). The water mass communities could only oxidize 23 of the 95 carbon sources. The diversity of utilizable carbon sources was reflected in the community bionumbers (Table 1). The use of some carbon sources were more variable than others among the bacterial communities (Table 2).

Complex compounds, like the detergents Tween 80 and Tween 40, were oxidized most efficiently by SML bacterial communities from *M. annularis*, followed by *Diploria strigosa* (Table 2). Metabolism of Tween 80 was also significant with the *D. labyrinthiformis* community. Glycogen was utilized primarily by the *M. annularis* community, weakly fol-

Table 2: Utilization (absorbance over controls at 490nm) of various carbon sources by the SML bacterial communities after 72h from *M. annularis* (MA), *M. cavernosa* (MC), *D. labyrinthiformis* (DPL), *D. clivosa* (DPC), *D. strigosa* (DPS), *D. stokesii* (DST), *P. divaricata* (PD), *P. furcata* (PF), *A. agaricites* (AGA), *A. cervicornis* (AC), *A. palmata* (AP), Water Mass (WC). D-GLAL = D-galactonic acid lactone; D-GLA = D-galacturonic acid; p-HPAA = p-hydroxyphenylacetic acid; a-KBA = alpha-ketobutyric acid.

	MA	MC	DPL	DPC	DPS	DST	PD	PF	AGA	AC	AP	WC
tween 80	321	82	187	25	225	23	72	26	29	96	47	45
tween 40	323	54	119	92	202	121	104	109	54	111	77	36
L-arabinose	325	277	150	115	159	133	240	63	75	185	48	33
glycogen	286	-74	59	15	125	102	64	24	3	33	72	21
D-fructose	297	64	85	17	223	132	69	92	18	32	47	42
L-fucose	398	123	89	64	191	155	62	102	25	46	40	27
D-mannose	221	30	72	37	123	112	53	13	27	43	40	16
maltose	164	34	15	-5	104	70	25	8	-21	23	4	0
D-arabitol	257	53	42	28	102	95	-19	84	11	59	15	18
D-mannitol	211	39	69	47	119	141	34	15	8	13	43	16
D-erythritol	278	31	3	16	82	-35	24	6	0	28	-24	7
D-GLAL	393	180	238	-36	219	263	-22	135	-57	122	110	154
propionic acid	533	102	178	61	278	205	173	75	44	145	-14	24
D-GLA	495	119	97	67	182	112	179	39	-1	59	-3	-18
formic acid	129	41	64	0	77	100	43	-9	-17	26	-2	19
p-HPAA	37	-3	20	-13	-27	24	31	38	-8	16	-15	5
itaconic acid	-16	47	68	35	125	122	37	26	-40	69	-9	21
quinic acid	117	23	81	-11	233	110	41	36	3	32	4	55
a-KBA	87	92	110	61	191	57	27	60	23	85	9	28
malonic acid	-5	10	13	-7	59	-36	-11	-7	-41	66	-35	-61

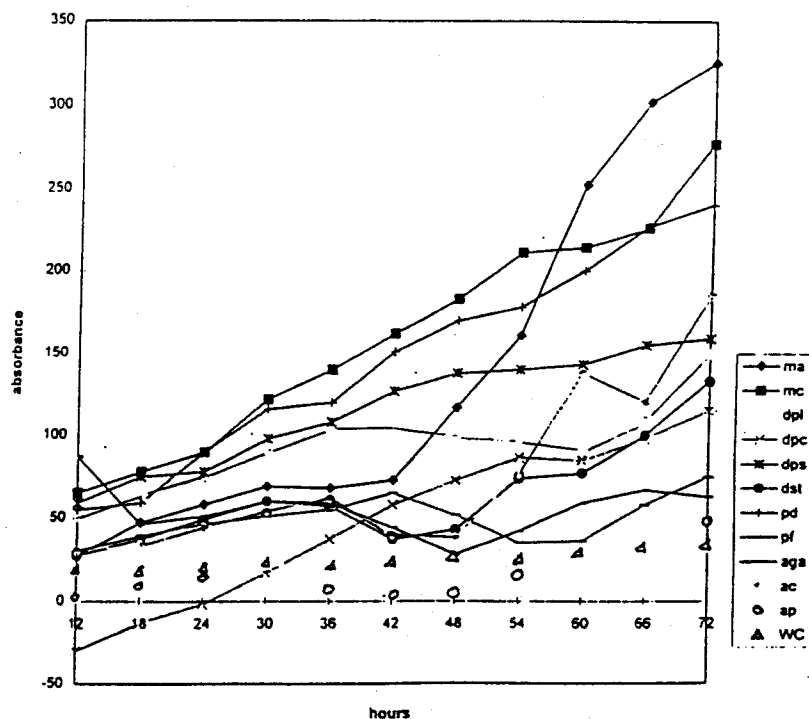


Fig. 1: Metabolism of L-arabinose by bacterial communities from various corals. ma: *Montasteria annularis*, mc: *M. cavernosa*, dpl: *Diploria labyrinthiformis*, dpc: *D. clivosa*, dps: *D. strigosa*, dst: *D. stokesii*, pd: *Porites divaricata*, pf: *P. furcata*, aga: *Agaricia agaricites*, ac: *A. cervicornis*, ap: *A. palmata*, WC: water mass.

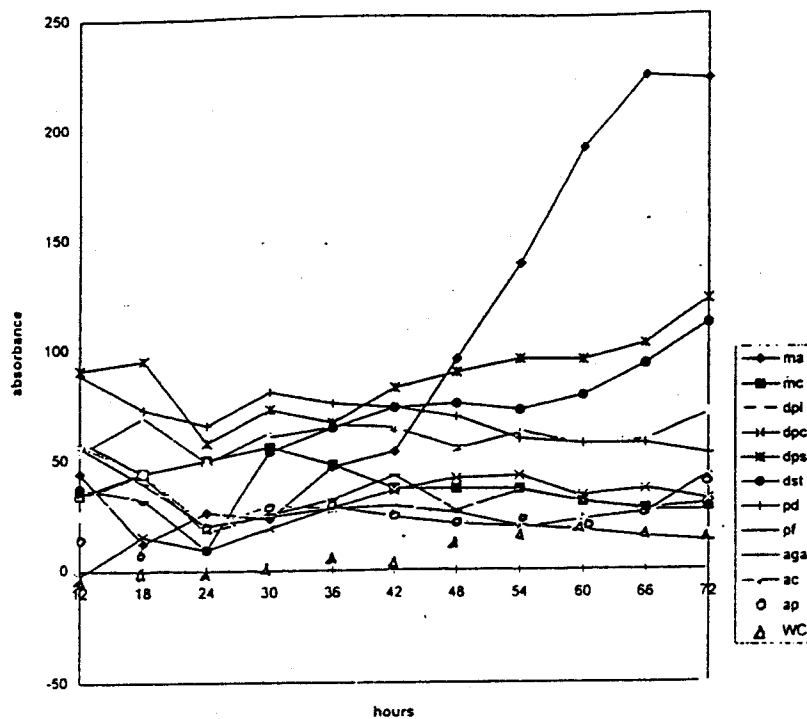


Fig. 2: Metabolism of the sugar D-mannose by bacterial communities. ma: *Montasteria annularis*, mc: *M. cavernosa*, dpl: *Diploria labyrinthiformis*, dpc: *D. clivosa*, dps: *D. strigosa*, dst: *Dichocoenia stoksi*, pd: *Porites divaricata*, pf: *P. furcata*, aga: *Agaricia agaricites*, ac: *Acropora cervicornis*, ap: *A. palmata*, WC: water mass.

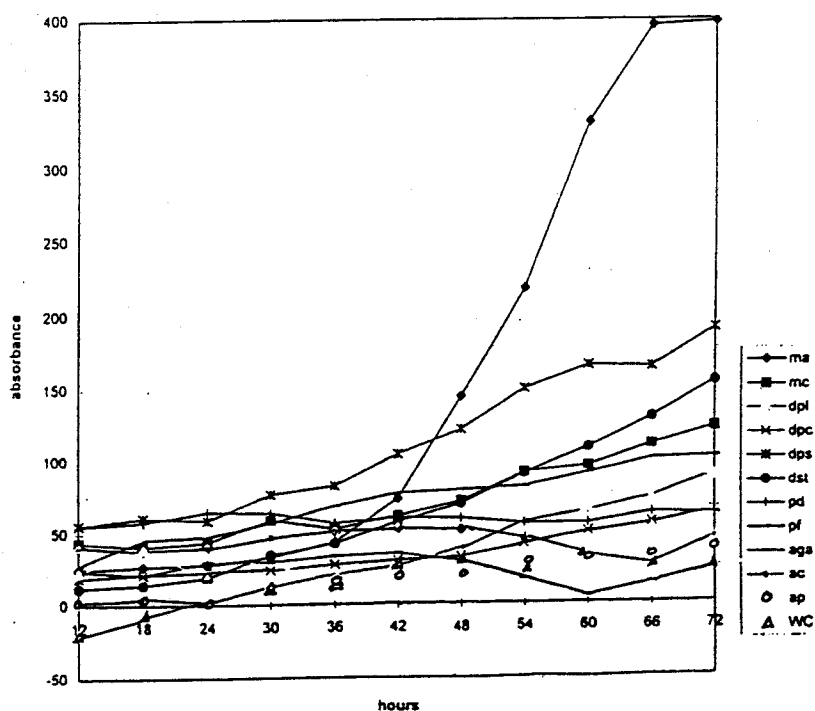


Fig. 1: Metabolism of L-fucose by bacterial communities. ma: *Montasteria annularis*, mc: *M. cavernosa*, dpl: *Diploria labyrinthiformis*, dpc: *D. clivosa*, dps: *D. strigosa*, dst: *Dichocoenia stoksi*, pd: *Porites divaricata*, pf: *P. furcata*, aga: *Agaricia agaricites*, ac: *Acropora cervicornis*, ap: *A. palmata*, WC: water mass.

Caledonian lagoonal reef. Proc 4th Int Coral Reef Symp 2:59-67

Disalvo LH (1971) Regenerative functions and microbial ecology of coral reefs: labelled bacteria in a coral reef microcosm. J Exp Mar Biol Ecol 7:123-136

Ducklow HW, Mitchell R (1979a) Composition of mucus released by coral reef coelenterates. Limnol Oceanogr 24:706-714

Ducklow HW, Mitchell R (1979b) Bacterial populations and adaptations in the mucus layers on living corals. Limnol Oceanogr 24:715-725

Herndl GJ, Velimirov B (1986) Microheterotrophic utilization of mucus released by the Mediterranean coral *Cladocora cespitosa*. Mar Biol 90:363-369

Hubbard JA, Pocock YP (1972) Sediment rejection by recent scleractinian corals: a key to paleoenvironmental reconstruction. Geol Rundsch 61:598-626

Means JC, Sigleo AC (1986) Contribution of coral reef mucus to the colloidal organic pool in the vicinity of Discovery Bay, Jamaica, WI. Bull Mar Sci 39:110-118

Meikle P, Richards GN, Yellowlees D (1988) Structural investigations on the mucus from six species of coral. Mar Biol 99:187-193

Pascal H, Vacelet E (1982) Bacterial utilization of mucus on the coral reef of Aqaba (Red Sea). Proc 4th Int Coral Reef Symp 1:669-677

Patton JS, Abraham S, Benson AA (1977) Lipogenesis in the intact coral *Pocillopora claptata* and its isolated zooxanthellae: evidence for a light-driven carbon cycle between symbiont and host. Mar Biol 44:235-247

Ritchie KB, Smith GW (1995a) Carbon-source utilization patterns of coral-associated marine heterotrophs. J Mar Biotechnol 3:105-107

Ritchie KB, Smith GW (1995b) Preferential carbon utilization by surface bacterial communities from water mass, normal and white-band diseased *Acropora cervicornis*. Mol Mar Biol Biotechnol 4:345-352

Ritchie KB, Dennis JH, McGrath TA, Smith GW (1994a) Bacteria associated with bleached and nonbleached areas of *Montastrea annularis*. Proc 5th Symp Nat Hist Bahamas:75-79

Ritchie KB, Smith GW, Gerace DT (1994b) Grouping of bacterial heterotrophs from scleractinian corals using metabolic potentials. 26th Meet Assoc Mar Labs Carib: 224-234

Ritchie KB, Jindal A, Hayes RL, Goreau TJ, Smith GW (1996a) Bacterial ecology of selected corals following the 1994 South Central Pacific bleaching event. 27th Meet Assoc Mar Labs Carib:26-32

Ritchie KB, Holley RA, McGrath TA, Smith GW (1996b) INT-linked dehydrogenase activity in the surface mucopolysaccharide layers of Bahamian scleractinian corals. Proc 6th Symp Nat Hist Bahamas:134-138

Segel LA, Ducklow HW (1982) A theoretical investigation into the influence of sublethal stresses on coral-bacterial ecosystem dynamics. Bull Mar Sci 32:919-935

Sorokin YI (1973) Trophical role of bacteria in the ecosystem of the coral reef. Nature 242:415-417

Smith GW, Hayasaka SS (1982) Nitrogenase activity associated with *Halodule wrightii* roots. Appl Environ Microbiol 43:1244-1248