PROCEEDINGS OF THE SEVENTH SYMPOSIUM ON THE NATURAL HISTORY OF THE BAHAMAS

Edited by
Tom K. Wilson

Conference Organizer
Kenneth C. Buchan

Bahamian Field Station, Ltd.
San Salvador, Bahamas
1998

Cover Illustration: ArcView GIS generated elevation map of San Salvador. Produced by Matt Robinson of the University of New Haven for the Bahamian Field Station

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Printed in USA by RSMAS, University of Miami, Miami, Florida

ISBN 0-935909-66-4

METABOLISM OF NITROGENOUS COMPOUNDS BY THE SML BACTERIAL COMMUNITIES OF SCLERACTINIAN CORALS

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ABSTRACT

Bacterial Communities associated with the surface mucopolysaccharide layer (SML) of hard corals play an important nutritional role, as well as, a probable role in maintaining health and preventing disease in the coral animal. The composition of the mucus varies among the different coral species and may select for specific members of the bacterial community. We tested the abilities of the SML heterotrophic bacterial communities from 11 different coral species to utilize 27 nitrogenous substrates as sole carbon sources and monitored the utilization rates over a 72 hour period. In general, the SML communities from the boulder corals (particularly *Montastera annularis*) showed greater rates and higher amounts of oxidation of the 20 protein amino acids, although other communities could oxidize these to a lesser degree. Some compounds were generally nonstimulatory or lethal for most (although not all) communities. These included: urocanic acid, L-phenylalanine, glucuronamide, L-pyroglutamic acid, succinamic acid, 2-amino ethanol, and γ-amino butyric acid. The SML communities of hard corals do exhibit differential metabolic stimulation by different compounds. Therefore, the composition of the nitrogenous fraction of the coral mucus may influence the bacterial community structure occurring within the SML of specific coral species.

INTRODUCTION

The surface of the living corals are covered by surface mucopolysaccharide layers (SMLs), the primary purpose of which seems 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 coral species have shown these structures to be rich in 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 the 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 Vacelet 1981; Herndl and Velimirov 1986).

MATERIALS AND METHODS

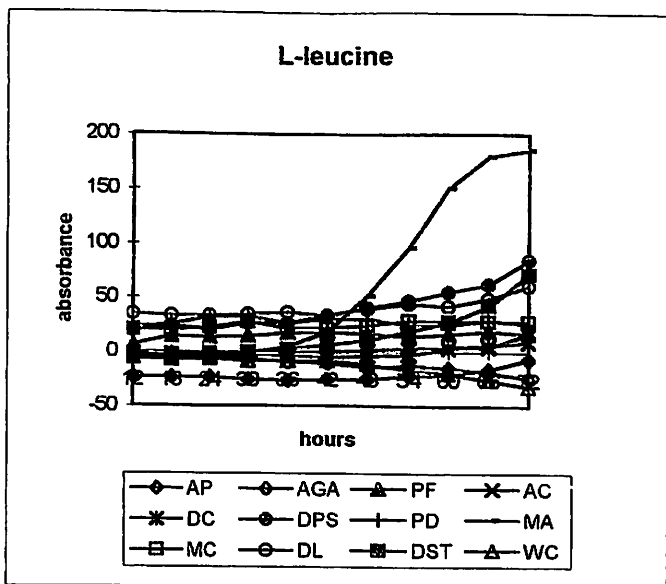
SMLs, containing the microbial communities, were collected from shallow (<5m) patch reefs in San Salvador, Bahamas (24°3'N by 74°30'W) using 30ml needeless sterile syringes. Triplicate samples of the following coral species were obtained: *Montastrea annularis; M. cavernosa; Diploria labyrinthiformis; D. strigosa; D. clivosa; Porites divaricata; P. furacata; Dichocoenia stokesii; Agaricia agaricites; Acropora cervicornis* and *A. palmata*. Water mass samples surrounding the coral were also taken for comparison. All samples were kept on ice or in a cold room at (4°C) until metabolic testing in the laboratory.

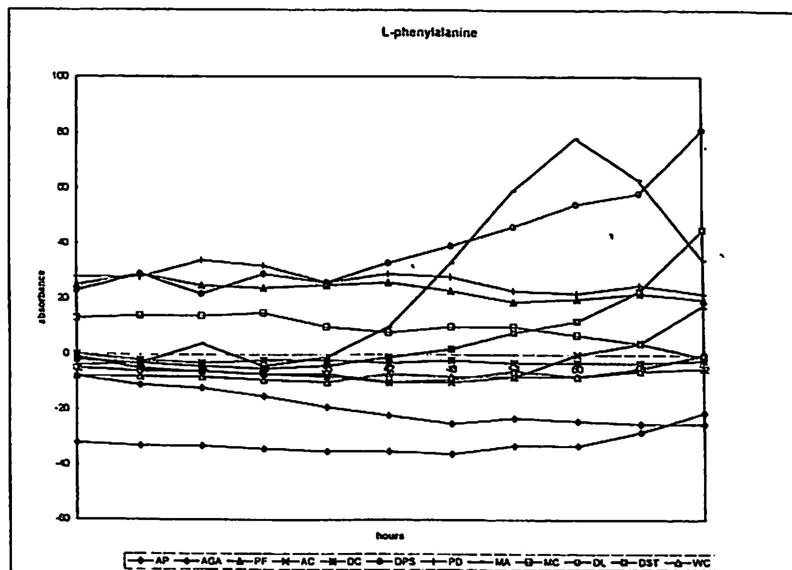
Syringes were allowed to equilibrate at room temperature for three hours after which subsample dilutions were spread plated on a nonspecific media (Smith and Hayasaka 1982). Samples were dispensed into Biolog GN microtiter plates (Bochner 1989) which contain 95 different potential carbon sources, each 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 after incubation at 25°C.

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 assign 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 the values assigned to sequential microwells in

Table 1: Utilization (%absorbance over controls at 490nm) of 27 nitrogenous carbon sources by the SML bacterial communities after 72h from M. annularis (MA), M. cavernosa (MC), D. labyrinthiformes (DPL), D. clivosa (DPC), D. strigosa (DPS), D. stokesii (DST), P. diverculata (PD), P. furcata (PF), A. agaricites (AGA), A. cervicornes (AC), A. palmata (AP), Water Mass (WC).

	MA	MC	DPL	DPC	DPS	DST	PD	PF	AGA	AC	WC
L-alanyl-glycine	671	293	322	198	378	344	284	188	172	228	107
L-alanine	725	289	236	118	368	356	226	224	174	260	112
D-alanine	296	130	125	57	233	142	122	139	35	74	69
L-aspartic acid	759	305	177	71	413	246	198	271	87	146	106
glycyl-L-aspartic acid	739	259	307	189	417	360	211	168	125	215	206
L-glutamic acid	747	270	231	94	307	326	154	127	105	217	176
glycyl-L-glutamic acid	600	203	295	181	480	273	191	125	100	176	122
L-asparagine	758	423	387	121	665	543	375	291	271	462	167
L-serine	637	279	200	91	322	342	229	178	133	175	110
D-serine	545	211	176	120	314	252	194	145	89	135	119
L-histidine	502	143	158	61	253	173	105	73	26	134	83
L-threonine	521	174	231	93	347	197	204	146	86	122	119
L-proline	407	181	163	98	244	213	135	95	65	136	66
hydroxy-L-proline	329	134	138	67	196	207	110	59	42	125	75
L-leucine	186	28	61	9	85	72	26	17	-24	17	-32
L-phenylalanine	34	-1	0	-2	81	45	22	20	-25	18	-5
L-ornithine	251	48	59	18	144	100	42	4	-2	65	52
D,L-carnitine	-17	14	-6	15	109	59	5	9	25	10	14
glucuronamide	251	81	100	32	193	127	81	31	-20	-29	39
urocanic acid	118	-10	8	-49	-41	-50	5	-59	-71	-39	-13
putrescine	125	18	67	18	134	2	32	13	-9	18	6
alaninamide	-1	47	10	-8	101	78	16	24	0	42	6
L-pyroglutamic acid	-9	28	-6	26	95	-9	45	-17	-27	24	-4
succinamic acid	22	20	9	24	117	59	34	17	6	49	8
2-amino ethanol	-19	30	14	-43	51	-20	7	-69	-32	-42	-9
γ-amino butyric acid	-15	-4	29	-13	162	86	51	42	-17	39	28
phenyl ethylamine	475	186	218	88	270	186	202	123	78	150	135





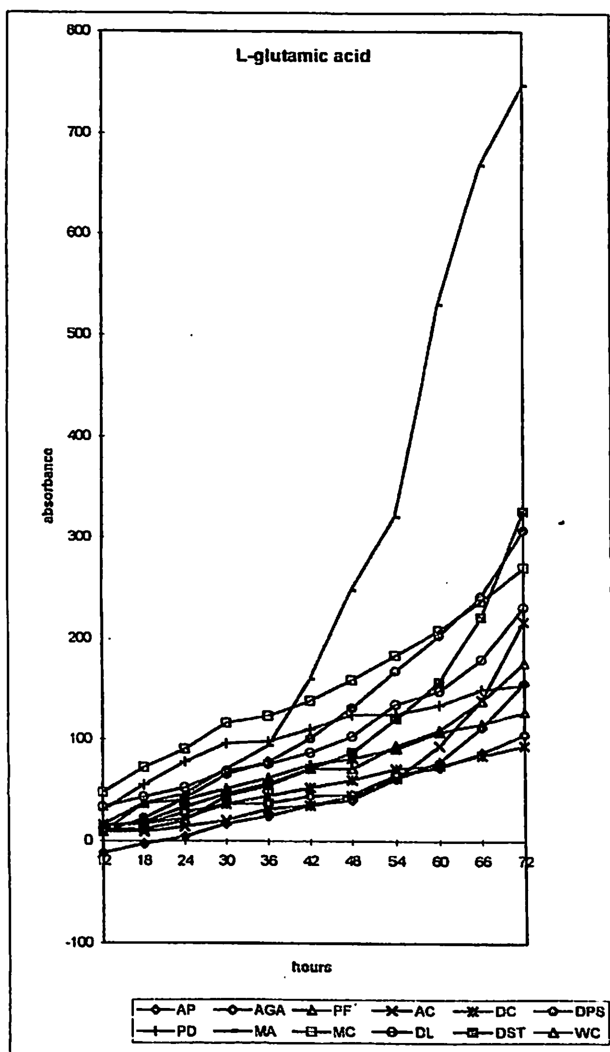
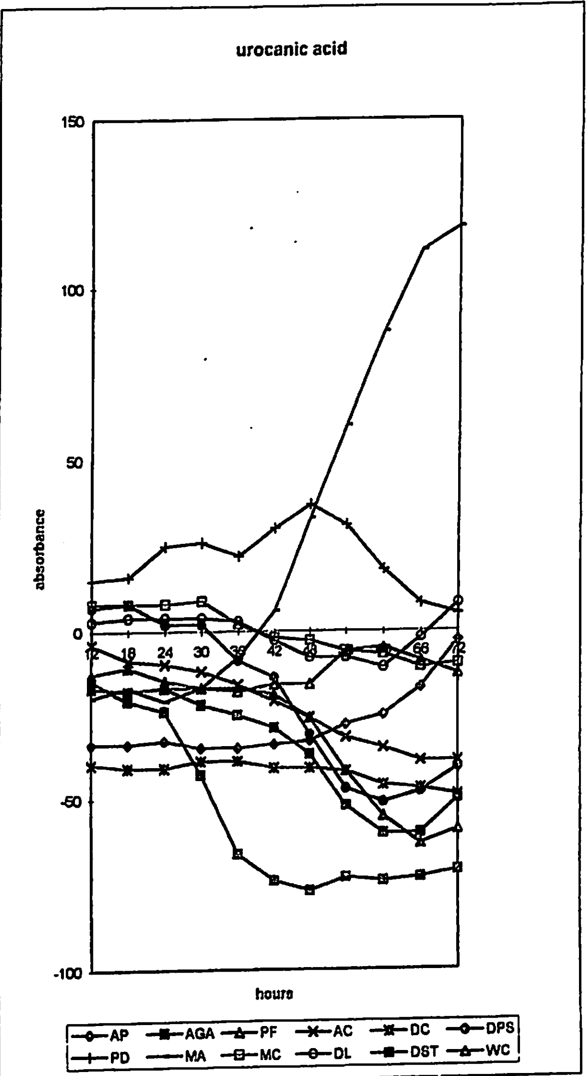


Figure 1 Metabolism of protein amino acids; L-leucine, L-phenylalanine, and L-glutamic acid by bacterial communities from various corals. AP: *Acropora palmata*, AGA: *Agaricia agaricites*, PF: *Porites furcata*, AC: *A. cervicornis*, DC: *Diploria clivosa*, DPS: *D. strigosa*, PD: *P. divaricata*, MA: *Montasteria annularis*, MC: *M. cavernosa*, DL: *D. labyrinthiformis*, DST: *Dichocoenia stokesii*, WC: water mass



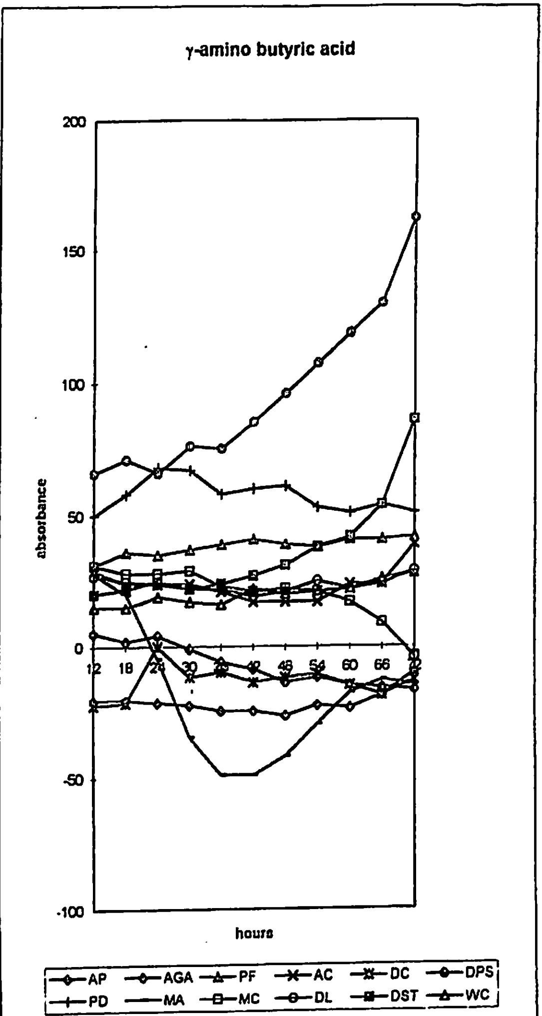


Figure 2 Metabolism of urocanic acid and □-amino butyric acid by bacterial communities from various corals. AP: *Acropora palmata*, AGA: *Agaricia agaricites*, PF: *Porites furcata*, AC: *A. cervicornis*, DC: *Diploria clivosa*, DPS: *D. strigosa*, PD: *P. divaricata*, MA: *Montasteria annularis*, MC: *M. cavernosa*, DL: *D. labyrinthiformis*, DST: *Dichocoenia stokesii*, WC: water mass.

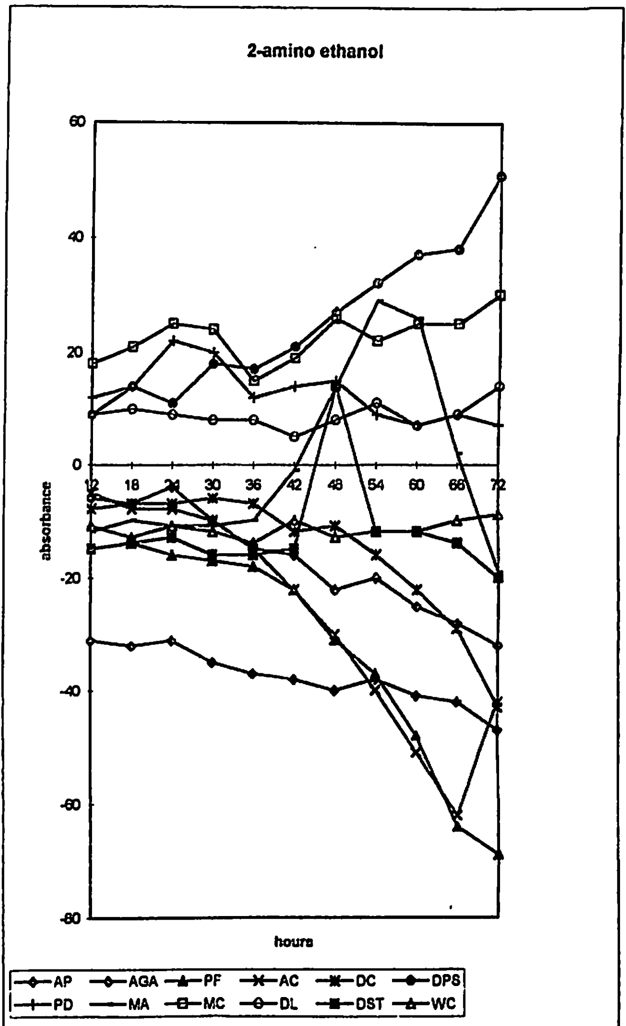


Figure 3 Metabolism of 2-amino ethanol by bacterial communities from various corals. AP: *Acropora palmata*, AGA: *Agaricia agaricites*, PF: *Porites furcata*, AC: *A. cervicornis*, DC: *Diploria clivosa*, DPS: *D. strigosa*, PD: *P. divaricata*, MA: *Montasteria annularis*, MC: *M. cavernosa*, DL: *D. labyrinthiformis*, DST: *Dichocoenia stokesii*, WC: water mass.

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 and oxidation trends of the nitrogenous carbon sources among the bacterial communities were evaluated and compared. All tests were run in triplicate. There was negligible variation for any particular coral species-nitrogenous carbon source combination.

RESULTS AND DISCUSSION

Bacterial communities removed from SMLs of the boulder corals (*M. annularis, M. cavernosa; D. labyrinthiformis; D. strigosa; D. clivosa)* demonstrated the highest oxidative activity of the nitrogenous carbon sources tested. Utilization of the 27 nitrogenous substrates was highest in the bacterial communities associated with *M. annularis.*

The more common nitrogenous compounds (common amino acids and their derivatives) showed the broadest metabolic degradation. The percent absorbance of the 27 nitrogenous compounds is shown in Table 1. Figure 1 shows the metabolism of L-leucine, L-phenylalanine, and L-glutamic acid. These figures are indicative of the metabolic rates for all the common amino acids. The bacterial communities associated with the *M.annularis*, *D.strigosa*, and D.*labyrinthiformis* have the highest metabolic rates. The other bacterial communities are able to break down these compounds – only at slower rates.

Metabolic rates of glycyl-L-glutamic acid, L-alanyl-glycine, L-alanine, D-alanine, L-aspartic acid, glycyl-L-aspartic acid, L-asparagine, L-serine, D-serine L-proline, hydroxy-L-proline, L-ornithine, L-histidine, L-threonine, phenyl ethylamine, glucuronamide, and putrescine were highest in *M. annularis*, *D. strigosa*, *D. stokesii*, and *D. labyrinthiformes*. The other groups showed similarly moderate rates of metabolic activity.

Metabolism of γ-amino butyric acid was highest in D. stokesii community. *M. annularis*, *A. agaricites*, *A. palmata*, and *D. clivosa* communities all showed a negligible metabolic activity. Presence of γ-amino butyric acid as the sole carbon source caused a decrease in percent absorbance over the 72 hour period for these SML communities. *M. annularis* communities were the only group to respond with a positive increase in percent absorbance. Like γ-amino butyric acid, the metabolic rates decreased over time (Figure 2).

D,L-carnitine and alaninamide metabolism was dominated by *D. strigosa* and *D. stokesii*. *M. annularis*, which was most often seen to dominate, along with the other communities appeared to have a negligible response to the presence of these two compounds. The percent absorbance either static or decreasing over the 72 hour period.

L-pyroglutamic acid and succinamic acid both had non-stimulatory effects on all communities except *D. strigosa*. The percent absorbance was generally static for other SML communities.

2-amino ethanol was not utilized at an appreciable rate by any of the SML bacterial communities. There was a quite noticeable decrease in percent absorbance for most of the coral SML communities. *D. strigosa* and *M. cavernosa* communities demonstrated only a slight increase in absorbance over the 72 hour period(Figure 3).

In summary, most of the metabolic activity was dominated by the boulder-type coral bacterial communities. As shown by previous studies (Ritchie and Smith 1997), the higher rates of metabolism by *M. annularis*, *D. strigosa*, *D. stokesii*, and *D. labyrinthiformes* coral SML bacterial communities are most likely a function of the variety and/or diversity of the carbon sources contained in the coral animal itself.

ACKNOWLEDGEMENTS

The coral species identification were made by Tom McGrath. This work was supported in part by the Bahamian Field Station and the Center for Field Research (Earthwatch).

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