Molecular Marine Biology and Biotechnology (1995) 4(4), 345–352

Preferential carbon utilization by surface bacterial communities from water mass, normal, and white-band diseased *Acropora cervicornis*

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Abstract

Bacterial heterotrophs were isolated from the water mass and from the surface mucopolysaccharide layers of normal *Acropora cervicornis* (staghorn coral) and *A. cervicornis* showing signs of white-band disease. Each isolate was exposed to 95 different carbon sources, and the percentages of isolates from each community were compared with respect to their ability to utilize each carbon source. Six-carbon sugars were preferentially metabolized by the white-band community while five-carbon sugars were preferentially used by the normal coral community. More organic and amino acids preferentially were oxidized by the white-band community over the other communities tested. In addition, pyrimidines, glycerol, and phosphorylated compounds also were preferential for the white-band community. This community was less diverse than other communities. These results support previous taxonomic comparisons and may yield insights into the overall pathogenesis of the disease.

Introduction

The important nutritional relation between scleractinian, or hard, corals and bacteria, particularly those residing in the surface mucopolysaccharide layers (SMLs), has been recognized for some time (Sorokin, 1973). Bacterial populations and overall microbial populations were found to be greater in the coral SML than in the water mass (Paul et al., 1986; Schiller and Herndl, 1989). Segel and Ducklow (1982) found that stress induced both an increase in mucus secretion by the corals and a concomitant increase in bacterial populations in the SML. Although few studies have been concerned with the structure of the bacterial communities associated with the SML of corals, Ritchie et al. (1994) showed a shift in the community structure from *Pseudomonas* spp. to *Vibrio* spp. when normal *Montastrea annularis* colonies became bleached.

Bleaching (loss of endosymbiotic zooxanthelle), among hard corals in general, appears to be increasing worldwide (Glynn, 1993). As a result, mass mortality of vast reef ecosystems can result. The cause of bleaching can vary, but increased temperatures are strongly correlated with mass bleaching events (Goreau and Hayes, 1994). A specific type of bleaching, referred to as white-band disease (WBD), appears to affect particularly the acroporid corals (Gladfelter et al. 1977; Gladfelter, 1982; Dustin, 1994), including *Acropora cervicornis* (Bak and Nieuwland, 1994). Peters et al. (1983) and Peters (1984) reported histologic evidence that WBD was associated with a bacterium. The bacteria appeared to form microcolonies within the coral tissue, perhaps replacing the zooxanthelle. Ritchie et al. (1995) isolated bacteria resembling *Vibrio charcharia* and *V. mediterranei* specifically from the SML of living, bleached *A. cervicornis* with WBD. These putative pathogens are being studied, but presently the pathogenesis of WBD is unknown. Figure 1 shows *A. cervicornis* demonstrating symptoms of WBD.

A. cervicornis is of particular interest because of its potential for transplantation into areas where other species have died out. *A. cervicornis* is a broadcast spawner and exhibits a comparatively fast growth rate (Gittings et al., 1994), but is susceptible to WBD and anthropogenic impacts (Sullivan et al., 1994). Die-offs of *A. cervicornis* have been reported throughout the Western Atlantic and Caribbean (Cortes, 1994; Ogden and Ogden, 1994) including the Bahamas (Curran et al., 1994; Dennis and Wickland, 1994).

Recently we performed a bacterial taxonomic comparison of the SMLs from normal and bleached corals, based on carbon source utilization patterns

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Figure 1. *A. cervicornis* showing bleaching (light areas), the first noticeable symptom of white-band disease.

(submitted). We found a decrease in species diversity associated with *A. cervicornis* showing symptoms of WBD compared with normal *A. cervicornis,* bleached *Montastrea annularis,* and normal *M. annularis.* As the amount of mucus increases when corals are stressed (Ducklow and Mitchell, 1979) and the heterotrophic bacterial SML community can use mucus as a carbon source (Herndl and Velimirov, 1986; Pascal and Vacelet, 1981), a shift in SML populations would indicate that stress induces a qualitative change in mucus composition, as well as a quantitative change. The purpose of this paper was to compare SML bacterial utilization of specific carbon sources among communities from the water mass, normal, and WBD *A. cervicornis.* We were particularly interested in which, if any, carbon sources were preferentially metabolized by a specific bacterial community.

Results and Discussion

Bacteria residing in SML should be exposed to a number of sugars. The percentages of the total heterotrophic culturable communities, from each source able to utilize the 33 different sugars or carbohydrates tested, are listed in Table 1. Setting an arbitrary limit of at least 10% increase in community usage as indicating preference, 18 of the sugars showed preferential utilization. Isolates from white-band diseased coral had a preference for eight carbohydrates, those from normal corals showed a preference for six, and four sugars were preferred by corals in general (regardless of disease status) over water mass isolates (Table 2). No preference for carbohydrates was detected by water mass isolates.

The WBD community preferred six-carbon monosaccharides, while the normal community preferred five-carbon sugars and the deoxysugar L-rhamnose. D-Sorbitol was the only monosaccharide preferred by both coral communities over the water mass isolates. Among the disaccharides, the WBD community preferred D-trehalose and sucrose, both commonly used in the synthesis of capsules and slime layers. This indicates that the increased mucopolysaccharide layers typical of bleached corals (Segal and Ducklow, 1982) may be synthesized by the bacterial community rather than the coral.

Disaccharide preference by the normal community included only lactulose, while α-D-lactose, cellobiose, and melibiose were preferred by the coral isolates in general. The trisaccharide D-raffinose was preferred by the normal community and glycogen by the WBD isolates.

Among the 28 organic acids tested, 10 exhibited preferential utilization by some community. Seven of these were preferred by the WBD community, one by normal coral isolates, and two by the water mass community (Table 3). No organic acid was preferred by coral isolates in general (regardless of disease status).

Preferred organic acids used as carbon sources are listed in Table 4. The WBD community preferred

Table 1. Percentage of isolates from the water mass, normal, and white-band diseased *A. cervicornis* utilizing various sugars and carbohydrates.

	Source of isolates		
Carbon source	Water mass	Normal coral	White-band disease
α-Cyclodextrin	20	37	28
Dextrin	68	62	72
Glycogen	63	53	74
Tween 40	76	72	85
Tween 80	68	68	71
N-acetyl-D-galactosamine	5	10	34
N-acetyl-D-glucosamine	68	67	86
Adonitol	5	12	9
L-Arabinose	5	12	2
D-Arabitol	5	17	0
Cellobiose	44	62	62
i-Erythritol	10	13	3
Fructose	95	80	91
L-Fucose	15	20	23
D-Galactose	51	67	83
Gentiobiose	49	52	60
α-D-Glucose	83	75	85
m-Inositol	22	35	34
α-D-Lactose	29	47	51
Lactulose	10	22	9
Maltose	73	72	82
D-Mannitol	61	67	80
D-Mannose	71	58	83
D-Melibiose	17	28	31
β-Methyl-D-glucoside	56	50	63
D-Psicose	46	47	43
D-Raffinose	7	18	8
L-Rhamnose	5	17	8
D-Sorbitol	27	38	40
Sucrose	63	67	78
D-Trehalose	73	67	85
Turanose	20	28	26
Xylitol	5	23	6

Table 2. Sugars and carbohydrates preferred by white-band disease, normal, and water mass *A. cervicornis* communities.

Source	Monosaccharides	Disaccharides	Trisaccharides	Polymers
White-band isolates	*N*-acetylgalactosamine *N*-acetylglucossamine D-Galactose D-Mannose D-Mannitol	D-Trehalose Sucrose		Glycogen
Normal coral isolates	L-Rhamnose L-Arabinose Xylitol D-Arabitol	Lactulose	D-Raffinose	
Water mass isolates	D-Sorbitol	α-D-Lactose Cellobiose Melibiose		

Table 3. Percentage of isolates from each source utilizing various organic acids.

Carbon source	Source of isolates		
	Water mass	Normal coral	White-band disease
Methyl pyruvate	56	60	77
Monomethyl succinate	5	2	0
Acetic acid	85	75	97
cis-Aconitic acid	68	57	75
Citric acid	61	53	77
Formic acid	7	3	0
D-Galactonic acid lactone	20	10	0
D-Galacturonic acid	5	12	3
D-Gluconic acid	59	60	71
D-Glucosaminic acid	5	12	8
D-Glucuronic acid	46	47	62
α-Hydroxybutyric acid	32	45	35
β-Hydroxybutyric acid	24	8	2
γ-Hydroxybutyric acid	5	7	0
p-Hydroxyphenylacetic acid	5	3	9
Itaconic acid	12	7	3
α-Keto butyric acid	54	53	42
α-Keto glutaric acid	46	53	48
α-Keto valeric acid	5	7	2
D,L-Lactic acid	73	77	89
Malonic acid	12	7	0
Propionic acid	85	82	91
Quinic acid	10	18	5
D-Saccharic acid	5	10	11
Sebacic acid	2	0	0
Succinic acid	66	67	91
Bromosuccinic acid	41	52	58
Succinamic acid	7	8	0

Table 4. Preference for organic acids by white-band disease, normal coral, and water mass communities.

White-band isolates	Normal coral isolates	Water mass isolates
Acetic	α-Hydroxybutyric	β-Hydroxybutyric
D,L-Lactic		D-Galacturonic
Methyl-pyruvate		
Succinic		
Citric		
D-Glucuronic		
D-Gluconic		

acetic, D,L-lactic, and methylpyruvic acids, which are all common substrates for sulfur-reducing bacteria. White-band isolates also preferred succinic and citric acids, which are intermediates in both glyoxylate and tricarboxylic acid cycles. In addition, D-glucuronic and D-gluconic acids were also preferred by the WBD community. Only α-hydroxybutyric acid was preferred by the normal community, and the water mass community showed a preference for β-hydroxybutyric and D-galacturonic (a component of pectin and agar) acids.

More than half of the amino acids tested (14 of 22) exhibited substrate preference by one of the coral communities (Table 5). Most preference was found with WBD isolates (11 amino acids). Two amino acids showed preference for normal coral isolates.

Table 5. Percentage of isolates from each source utilizing nitrogens carbon sources.

	Source of isolates		
Carbon source	Water mass	Normal coral	White-band disease
Glucuronamide	7	15	5
Alaninamide	27	33	18
D-Alanine	56	55	82
L-Alanine	76	85	94
L-Alanyl-glycine	66	72	91
L-Asparagine	83	72	85
L-Aspartic acid	66	57	78
L-Glutamic acid	76	72	86
Glycl-L-aspartic acid	66	62	85
Glycl-L-glutamic	63	67	86
L-Histidine	32	20	49
Hydroxy L-proline	32	48	46
L-Leucine	15	12	8
L-Ornithine	17	10	8
L-Phenylalanine	5	0	0
L-Proline	76	67	86
L-Pyroglutamic acid	15	20	0
D-Serine	37	50	68
L-Serine	71	73	88
L-Threonine	63	58	86
D,L-Carnitine	5	7	0
γ-Aminobutyric acid	2	12	0

Table 6. Amino acids and dipeptide preference for white-band disease isolates.

Polar	Nonpolar	Positive charge	Negative charge
L-Threonine	D-Alanine	L-Histidine	L-Aspartic
D-Serine	L-Proline		Glycyl-L-aspartic
L-serine	L-Alanyl-glycine		L-Glutamic
			Glycyl-L-glutamic

These were λ-aminobutyric acid (a neurotransmitter inhibitor) and 2,3-butanediol. Only hydroxyl L-proline showed preference, regardless of disease status. The rest were preferential for WBD isolates.

Those amino acids and dipeptides showing preference for WBD isolates are listed in Table 6 by charge or polarity. Only one positively charged amino acid (L-histidine) showed preference for WBD isolates, while four negatively charged amino acid and dipeptides showed preference. This may be misleading, however, as the dipeptides were negatively charged because of aspartic and glutamic residues. Polarity did not apparently affect preference among the WBD isolates.

Among the remaining carbon sources, one (2,3-butanediol) was preferential for the normal community (Table 7), while seven were preferential for WBD isolates. These included inosine, uridine, and thymidine, possibly nucleic acid degradation products. In addition, glycerol and all of the phosphorylated substrates were preferential for WBD isolates. This indicates that the WBD community may be well suited to utilize substrates resulting from the death of the coral animal.

Because a large number of carbon sources were preferential for WBD isolates, this community was less diverse (rich) than other communities (Table 8). Diversity measurements based on taxonomic, rather

Table 7. Percentage of isolates from each source utilizing various carbon sources.

Carbon source	Source of isolates		
	Water mass	Normal coral	White-band disease
Urocanic acid	2	0	2
Inosine	68	73	89
Uridine	68	65	88
Thymidine	56	57	80
Phenyl ethylamine	5	0	2
Putrescine	5	10	18
2-Amino ethanol	5	0	2
2,3-Butanediol	5	17	3
Glycerol	71	72	85
D,L-α-Glycerol phosphate	46	47	71
Glucose-L-phosphate	54	55	74
Glucose-6-phosphate	49	50	74

Table 8. Diversity indices of carbon sources utilization by water mass, normal, and white-band disease *A. cervicornis* SML communities.

Index	Bacterial community		
	Water mass	Normal coral	White-band disease
Richness*	1.58	1.46	1.27
Heterogeneity†	1.84	1.86	1.80
Evenness‡	0.93	0.95	0.94

*Menhinick Index
†Shannon (H') Index
‡Pielou (J') Index

than carbon source, differences show a decrease in richness, heterogeneity, and eveness compared with water mass and normal coral isolates (our unpublished results).

Previously observed shifts in taxonomic groups of bacteria, due to the development of white-band disease in *A. cervicornis* (Ritchie et al., 1995), are reflected by the preferential use of specific carbon sources by a higher percentage of the bacterial community. Analysis of carbon source usage may yield clues indicating certain aspects of the pathogenesis of white-band disease. We have shown that the bacterial community in the SML is different from normal SML and that certain carbon sources can be used by the white-band community preferentially over normal coral and water mass isolates.

Experimental Procedures

Sample collection

Surface mucopolysaccharide layers were removed from living *Acropora cervicornis* stands on patch reefs in shallow water (less than 2 m) using 3.0-ml needleless syringes. These reefs were located in the NW sector of Graham's Harbor, San Salvador Island, Bahamas (24°3'N by 74°30'W). At the time of sample collection, many of the *A. cervicornis* stands showed symptoms of white-band disease (Figure 1). The SMLs were removed from normal (pigmented) and bleached (living) tissue, as well as the surrounding water mass (approximately 0.25 m from the corals). Syringes were capped and placed in sample bags out of the water. On shore, the contents of syringes were transferred to 1.5-ml sterile labeled vials and kept on ice or in a cold room (2°C) until laboratory processing.

Strain isolation and testing

Vials were equilibrated and vortexed at room temperature, after which 0.1 and 0.01-ml subsamples were spread plated onto a glycerol artificial seawater medium (Smith and Hayasaka, 1982) and incubated at 30°C for three days. This medium was used because of its nonselective characteristics. All detectable colonies were restreaked until pure cultures were obtained. Pure cultures were removed by adding 2.0-ml sterile artificial seawater (ASW) to the plate into which colonies were suspended using a sterile bent glass rod. The suspension was transferred to test tubes containing 20.0-ml sterile