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Isolation, Characterization and Identification of Bacteria associated with Mucus of *Acropora cervicornis* Coral from Bidong Island, Terengganu, Malaysia

Murugan Kalimutho[1*], Aziz Ahmad[1] and Zaleha Kassim[2]

[1] Laboratory of Microbiology, Department of Biological Sciences, Faculty of Science and Technology, University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
* mceric_rugan@hotmail.com (corresponding author)
[2] Institute Aquaculture Tropica, AKUATROP, University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
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Abstract Marine bacteria associated with mucus of *Acropora cervicornis* coral of Bidong Island were successfully isolated and cultured on sucrose sea water agar (SSW). The bacteria were characterized by using selective culture media and biochemical assays. Four major groups of bacteria were obtained, γ-proteobacteria, ά-proteobacteria, high G+C gram positive bacteria, CFB group and unknowns. The coral mucus-associated bacteria strains were identified as *Pantoea dispersa, Pseudomonas* sp., *Enterobacter agglomerans, Cadacea darisae, Serratia plymuthica, Citrobacter youngae, Erwinia herbicola, Vibrio* sp., *Klebsielle pneumonia* subspecies *ozanae, Aeromonas caviae, Alteromonas putrefaciens, Serratia* sp., *Alteromonas* sp., *Moraxella* sp., *Photobacterium* sp., *Yersinia bercovieri, Vibrio metschnikovii, Acinetobacter* sp., *Yersinia entrocolitica, Brucella* sp., *Micrococcus* sp., *Micrococcus varians, Micrococcus roseus, Actinomyces* sp. and *Flavobacterium* sp.

Abstrak Bakteria marin yang bersekongkol dengan mukus karang *Acropora cervicornis* telah berjaya dipencil dan dikultur atas agar media 'sucrose sea water (SSW)'. Pencirian bakteria telah dilakukan dengan menggunakan pelbagai jenis media terpilih dan asei biokimia. Bakteria ini dapat dikumpulankan kepada empat kumpulan utama sebagai 'γ-proteobakteria' , 'ά-proteobakteria', 'high G+C' gram positif bakteria, kumpulan 'CFB' dan bakteria tidak dapat dikenal pasti. Bakteria-bakteria yang bersekongkol dengan mukus karang telah dikenal pasti sebagai *Pantoea dispersa, Pseudomonas* sp., *Enterobacter agglomerans, Cadacea darisae, Serratia plymuthica, Citrobacter youngae, Erwinia herbicola, Vibrio* sp., *Klebsielle pneumonia* subspecies *ozanae, Aeromonas caviae, Alteromonas putrefaciens, Serratia* sp., *Alteromonas* sp., *Moraxell* sp., *Photobacterium* sp., *Yersinia bercovieri, Vibrio metschnikovii, Acinetobacter* sp., *Yersinia entrocolitica, Brucella* sp., *Micrococcus* sp., *Micrococcus varians, Micrococcus roseus, Actinomyces* sp. dan *Flavobacterium* sp.

(Acropora cervicornis, CMAB, Bidong Island, sucrose sea water agar, coral mucus)

INTRODUCTION

Coral reefs are the most diverse of all marine ecosystems and most of them remain uncharacterized [1, 2, 3]. Coral harbors diverse and abundant bacterial communities [4] and Archea [5]. These corals depend heavily on bacteria in all manner of action, including dissolved organic matter, immunity, involved in carbon and nitrogen cycle [6, 7]. According to Borneman [7] these bacteria are also responsible for the primary production of reefs. The bacteria present in the coral reef community can work on particulate matter, dissolved organic matter, and even the mucus itself to potentially change some of the substances into forms more usable by the coral. The large number of bacteria in coral mucus may act as a 'lure', attracting zooplankton than can be captured by corals [8]. These microorganisms protect corals from pathogens by blocking and/or by producing antibiotics or enhance the ability of corals to defend themselves

against predators or competitors [6]. Coral mucus was thought to play a major role in reef metabolism, as an important source of organic material supporting a high bacterial activity [9]. Until today, reports on the bacterial communities of healthy corals are very limited especially on *Acropora.* Unraveling the nature of these associations would be a difficult task due to the diverse biochemical capabilities.

Acropora cervicornis is a type of staghorn coral distributed along South Pacific Ocean. This type of coral is the most dominant and frequently found in epi-pelagic zone of Bidong Island, South China Sea. This is the first paper on the identification of coral mucus-associated bacteria of *A. cervicornis* from Bidong Island, Terengganu, Malaysia. The identified bacteria can be a starting material for novel metabolites which may have high potential in biotechnological and pharmaceutical applications.

MATERIALS AND METHODS

Sampling

A. cervicornis was obtained by diving to 4 - 5 meter depth in three different locations at North of Bidong Island, Terengganu. The coral branches were taken out and rinsed with filter sterilized seawater (0.4 μm). The coral spike was broken and 2 ml of the mucus released by coral were drawn into a sterile universal bottle containing sucrose sea water (SSW) (30g/l sucrose, 1g/l Yeast, 5g/l peptone, all chemicals were dissolved in seawater) and kept in an ice box for further analysis.

Isolation of the bacteria

Samples were taken out from icebox and incubated at room temperature for 24 hours and considered as stock culture. A series of dilutions were made from the broth of stock culture. A volume of 100 μl of diluted culture was spread on SSW medium plate prior to incubation at 28°C overnight. Single bacterial colonies with different morphological characteristics such as colony elevation, color, shape, margin and surface texture were isolated and transferred onto fresh SSW agar plates. The purified isolates were then subcultured onto 1 ml of SSW [10] and incubated at 28°C overnight with shaking at 100 rpm. The bacterial broth was then diluted with a sterile glycerol solution to the final concentration of 60% glycerol prior frozen at -21°C for archival purposes.

Morphological Characterization

Bacterial cultures grown on SSW agar were examined based on their Gram reaction by conventional staining techniques [11]. A series of selective mediums which are MacConkey agar, TCBS agar, Pseudomonas agar, Simmons Citrate agar, Eosin Methylene Blue agar (EMB) and salmonella agar were used to characterize these isolates. Motility test was performed using modified SIM medium containing filter sterilized seawater [12].

Phenotypic characterization

Isolated strains were characterized by conventional microbiological methods [13, 14] involving following characteristics assays: Catalase Test; Oxidase Test; Nitrate Reduction Test; Methyl Red Test; Voges-Proskauer Test; Indole Production Test; HL media (O/F); degradation of starch, urea, casein, Tween-20, Tween-80, gelatin; gas and acid production from D-lactose, D-galactose, D-sucrose, D-arabinose, D-maltose, D-fructose, D-mannitol, dextrose and Myo-inositol; utilization of citrate and propionate; blood hemolysis; bioluminescence; Triple Sugar Iron Test; growth temperature (4, 28, 37, 40, 50, or 60°C) and present of NaCl (0, 3, 6, 9, 12, 15 or 20%). In this assays, the bacteria were grown on the specific medium according to the standard preparation protocol with minor modification. All media used were added with filtered-sterilized seawater using nitrocellulose membrane to fulfill the halophilic requirement of marine bacteria. The pH was adjusted according to the type of media used. *Escherichia coli* and *Bacillus* sp. were used as a control.

Identification of the isolates

The bacteria were identified according to Bergey's Manual of Determinative Bacteriology (10th Edition) and Probabilistic identification of Bacteria for Windows (PIBWin Programme), which can be accessed from http://www.som.soton.ac.uk/staff/tnb/pib.htm.

RESULTS AND DISCUSSION

The bacteria associated with mucus of coral, *A. cervicornis* from sea of Bidong Island Terengganu, Malaysia were successfully isolated and characterized. These marine bacteria can be isolated using standard culture methods. By

inoculating coral mucus into culture media, it has shown that corals harbor diverse and abundant bacterial communities. A total of 30 isolates which demonstrated some conspicuous attributes in their conventional test results and which were presumptively identified on the basis of a biochemical profile most closely resembling that of a particular species and genus were recovered (Table 1).

Cultural and biochemical characteristics of the entire isolates and Gram reaction are variable. The majority of the bacteria lie in the Gram-negative category. This was similar with previous finding by Macleod [15, 16], of which 87% of total bacteria in *A. cervicornis* were Gram negative. Meanwhile, soils of terrestrial environment containing only 27 - 36% of the Gram negative bacteria [17]. The motility test shown that some of the bacteria are motile with the presence of flagella. Tentatively, fifteen isolates were identified until species level, twelve isolates were identified until genus level and three isolates were categorized as unknown species. The bacteria can be divided into four major groups which are γ-proteobacteria, ά-proteobacteria, High G+C Gram positive bacteria, CFB group and the unknown status. The ά -Proteobacteria, γ -Proteobacteria and CFB group bacteria were reported as dominant groups in the marine environment and marine bio-films [18]. With regards to the systematic position, the coral mucus-associated bacteria (CMAB) belonged to numerous families and genera as in terrestrial. The only difference between CMAB and closely related terrestrial form with identical metabolic reaction is salt tolerance (halophilic) and the facultative psychophilic character of the marine form. Often, the marine, soil and freshwater bacteria are grouped together in same genus. The ability to live in the sea is the only characteristic which clearly distinguishes them from other bacteria, this one characteristic is nevertheless sufficient to delimit them because under natural conditions both marine and terrestrial bacteria might have developed from original marine ancestors [15, 16].

The bacteria may also play a crucial role to coral metabolisms [19], which these bacteria compose, an important tropic role in the heterotrophic needs of corals. There is strong relationship between the mucus and the bacteria since the mucus is an extremely good medium for bacteria growth [4]. The level of bacterial productivity in coral mucus is at least one order greater than in the surrounding water and ever found levels on the coral surface to be seven times higher [6].

It has been hypothesized that marine bacteria associated with invertebrates and vertebrates secrete a number of antibacterial agents that may provide a level of immunity to the corals [20]. This is commonly known among us as 'probiotic'. Marine bacteria were found having a capacity to produce the antimicrobial compound compared with terrestrial microorganisms [20]. The screening process and use marine bacteria for the production of antibiotic in pharmacological industries are increasing tremendously.

As a conclusion, phenotypic of biodiversity studies of microbial communities of *A. cervicornis* mucus has provided valuable information on the existence of potential known bacteria. There are still many opportunities for new discoveries in this coral mucus studies, and the results have also opened new questions about the activities of these bacteria and their function, going beyond just listing taxa. Rarely can the broad function be inferred from phylogenetic position alone.

Table 1. Morphological and biochemical characterization of bacteria isolated from mucus of *Acropora cervicornis*

CHARACTERISTICS		MD001	MD004	MD005	MD006	MD007	MD008	MD009	MD011	MD012
Morphology of organisms		Coccobacilli	Coccus	Rod	Coccobacilli	Coccus	Coccus	Rod	Short Rod	Coccobacilli
Colony color		Yellowish Cream	Yellowish	Orange	White	Orange	Yellowish	Whitish	Creamy	Yellowish
Gram's stain reaction		Negative	Positive	Negative	Negative	Positive	Positive	Positive	Negative	Negative
Motility		+	+	-	+	+	-	-	+	+
Catalase activity		+	+	+	+	+	+	-	+	+
Oxidase activity		-	-	-	-	-	-	+	-	-
HL media (O/F)		F	NA	NA	F	NA	NA	NA	F	F
VP test		-	-	-	-	-	-	-	-	-
MR test		+	+	+	+	+/-	+	-	+	+
Indole production		-	-	-	-	-	-	-	-	-
Growth on MacConkey agar		-	-	-	+ F	-	-	+NF	+NF	-
Growth on TCBS agar		-	-	-	-	-	-	-	-	-
Growth on Pseudomonas agar		-	-	-	-	-	-	-	-	-
Eosin Methylene Blue agar (EMB)		-	+ Pink	+ Pink	+	+ Pink	+ Pink	+ Pink	+Pink	+ Pink
Growth on Salmonella agar		+ Blue	-	-	-	-	-	-	-	+ Brown
Simmon citrate utilization		+	-	-	-	-	-	-	-	-
TSI (Triple Sugar Iron)	Butt (Glucose)	Y	Y/R	R	Y	R	R/Y	R	Y	Y
	Gas production	+	-	-	-	-	-	-	+	+
	H_2S production	-	-	-	-	-	-	-	-	-
	Slope (Lactose)	Y	Y	Y	Y	Y	Y	R	R/Y	Y
Casein hydrolysis		-	+	+	-	-	-	-	-	-
Nitrate reduction		+	+	+	+	+	+	NG	+	+
Starch Hydrolysis		-	+	-	+	-	-	-	-	-
Gelatin hydrolysis		-	-	-	-	-	-	-	-	-
Tween-20 hydrolysis		-	+	+	-	-	+	-	-	-
Tween-80 hydrolysis		-	+	-	-	-	+	-	-	-
Bioluminescence		-	-	-	-	-	-	-	-	-

Key: (+): Positive, (-): Negative, ND: Not done/determine, Y: Yellow, R: Red, +/-:Orange ,NG: No growth, A/G: Acid/Gas, A/-:Acid/No gas, -/-:not acid production/no gas ,NA: No Action , RT: Room Temperature, NF: Non-Fermented ,F: Fermented, HL: Hugh & Leifson, MR: Methyl Red, H_2S: Hydrogen Sulfate, NaCl: Sodium Chloride, NG: No Growth

Table 1. Morphological and biochemical characterization of bacteria isolated from mucus of *Acropora cervicornis* (continued)

CHARACTERISTICS		MD001	MD004	MD005	MD006	MD007	MD008	MD009	MD011	MD012
NaCl Requirement	NA without NaCl	+	+	+	+	+	+	+	+	+
	3 % NaCl	+	+	+	+	+	+	+	+	+
	6 % NaCl	+	+	+	+	+	+	+	+	+
	9 % NaCl	-	+	+	+	+	+	+	-	-
	12 % NaCl	-	+	+	+	+	+	-	-	-
	15 % NaCl	-	-	+	-	+	+	-	-	-
	20 % NaCl	-	-	-	-	+ Weak	+ Weak	-	-	-
Growth on different temperature	4 ° C	-	-	-	-	-	-	-	-	-
	RT	+	+	+	+	+	+	+	+	+
	37 ° C	+	+	+	+	+	+	+	+	+
	40 ° C	+	+	+	+	+	+	+	+	+
	50 ° C	-	-	-	-	+	+	-	-	-
	60 ° C	-	-	-	-	-	-	-	-	-
Urease activity		-	-	-	-	-	-	-	-	-
Blood hemolysis		-γ	-γ	-γ	-γ	-γ	-γ	-γ	-γ	-γ
Utilization of	Glucose	A/G	A/G	-/-	A/G	-/-	A/-	-/-	A/G	A/G
	Lactose	-/-	-/-	-/-	A/G	-/-	-/-	-/-	-/-	-/-
	Galactose	A/G	-/-	A/-	A/G	-/-	A/-	-/-	A/G	A/G
	Sucrose	A/G	A/-	-/-	A/G	A/-	A/-	ND	A/G	A/G
	D-Arabinose	A/G	-/-	-/-	A/G	A/-	-/-	ND	A/G	A/G
	D-Maltose	A/G	A/-	-/-	A/-	-/-	A/-	ND	A/G	A/G
	D-Fructose	A/G	A/-	A/-	A/-	A/-	A/-	-/-	A/G	A/G
	D-Mannitol	A/G	A/-	A/-	A/-	A/-	A/-	-/-	A/G	A/G
	Dextrose	A/G	A/-	-/-	A/G	-/-	A/-	-/-	A/-	A/G
	Myo-inositol	-/-	-/-	/-/	A/G	-/-	-/-	-/-	-/-	-/-
Lipid hydrolysis		+	-	+	+	+	+ weak	-	+	+
Tentatively identified genus		Pantoea	Micrococcus	Pseudomonas	Entrobacter	Micrococcus	Micrococcus	Actinomyces	Cadacea	Serratia
Tentatively identified species		*Pantoea dispersa*	*Micrococcus* sp.	*Pseudomonas* sp.	*Entrobacter agglomerans*	*Micrococcus roseus*	*Micrococcus varians*	*Actinomyces* sp.	*Cadacea darisae*	*Serratia plymuthica*

Key: (+): Positive, (-): Negative, ND: Not done/determine, Y: Yellow, R: Red, +/-:Orange ,NG: No growth, A/G: Acid/Gas, A/-:Acid/No gas, -/-:not acid production/no gas ,NA: No Action , RT: Room Temperature, NF: Non-Fermented ,F: Fermented, HL: Hugh & Leifson, MR: Methyl Red, H_2S: Hydrogen Sulfate, NaCl: Sodium Chloride, NG: No Growth

Table 1. Morphological and biochemical characterization of bacteria isolated from mucus of *Acropora cervicornis* (continued)

CHARACTERISTICS		MD014	MD017	MD018	MD019	MD021	MD022	MD024	MD025
Morphology of organisms		Short Rod	Coccobacilli	S.Coccobacilli	Coccobacilli	Coccobacilli	Rod	Coccobacilli	Coccobacilli
Colony color		Creamy	Whitish	Brownish	Brownish	Colourless	Yellowish	Whitish Blue	Whitish Blue
Gram's stain		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Motility		-	-	-	+	+	-	-	-
Catalase activity		+	+	+	+	+	+	+	+
Oxidase activity		-	-	-	-	-	-	-	-
HL media (O/F)		F	F	F	F	F	F	F	F
VP test		-	-	-	-	-	-	-	-
MR test		+	+	+	+	+	+	+	+
Indole production		-	-	-	-	-	-	-	-
Growth on MacConkey agar		-	-	-	+ F	+ F	+ F	+ F	+ F
Growth on TCBS agar		-	-	-	-	+ Yellow	-	+ Green	+ Green
Growth on Pseudomonas agar		-	-	-	-	-	-	-	-
Eosin Methylene Blue agar (EMB)		+	+ Pink	+ Pink	-	+ Pink	+ Pink	+	+ Pink
Growth on Salmonella agar		-	-	+ Colourless	-	+ Brown	+ Brown	-	-
Simmon citrate utilization		+	+	+	+	+	+	-	-
TSI (Triple Sugar Iron)	Butt (Glucose)	Y	Y	Y	Y	Y	Y	Y	Y
	Gas production	+	+	+	+	+	-	+	+
	H_2S production	-	-	-	-	-	-	-	-
	Slope (Lactose)	Y	Y	Y	Y	R/Y	Y	R/Y	Y
Casein hydrolysis		-	-	-	+	-	-	-	-
Nitrate reduction		+	+	+	+	+	+	+	+
Starch Hydrolysis		-	-	-	-	+	+	-	-
Gelatin hydrolysis		-	-	-	-	-	-	-	-
Tween-20 hydrolysis		-	-	-	-	-	+	-	-
Tween-80 hydrolysis		-	-	-	-	+	+	-	-
Bioluminescent		-	-	-	-	-	-	-	-
NaCl Requirement	NA without NaCl	+	+	+	+	+	-	+	+
	3 % NaCl	+	+	+	+	+	+	+	+
	6 % NaCl	+	+	+	+	+	+	+	+
	9 % NaCl	-	-	-	-	-	-	-	-
	12 % NaCl	-	-	-	-	-	-	-	-
	15 % NaCl	-	-	-	-	-	-	-	-
	20 % NaCl	-	-	-	-	-	-	-	-

Key: (+): Positive, (-): Negative, ND: Not done/determine, Y: Yellow, R: Red, +/-:Orange ,NG: No growth, A/G: Acid/Gas, A/-:Acid/No gas, -/-:not acid production/no gas ,NA: No Action , RT: Room Temperature, NF: Non-Fermented ,F: Fermented, HL: Hugh & Leifson, MR: Methyl Red, H_2S: Hydrogen Sulfate, NaCl: Sodium Chloride, NG: No Growth

Table 1. Morphological and biochemical characterization of bacteria isolated from mucus of *Acropora cervicornis* (continued)

CHARACTERISTICS		MD014	MD017	MD018	MD019	MD021	MD022	MD024	MD025
Growth on different temperature	4 ° C	-	-	-	-	-	-	-	-
	RT	+	+	+	+	+	+	+	+
	37 ° C	+	+	+	+	+	+	+	+
	40 ° C	+	+	+	+	+	+	+	+
	50 ° C	-	-	-	-	-	-	-	-
	60 ° C	-	-	-	-	-	-	-	-
Urease activity		-	-	-	-	-	-	-	-
Blood hemolysis		-γ	-γ	-γ	-γ	+ά	+ά	-γ	-γ
Utilization of	Glucose	A/G	A/G	A/G	A/G	A/-	A/G	A/G	A/-
	Lactose	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	Galactose	A/G	A/G	A/G	A/G	-/-	A/G	A/G	A/G
	Sucrose	A/G	A/G	A/G	A/G	A/-	A/G	A/G	A/G
	D-Arabinose	A/G	A/G	A/G	A/G	A/-	A/G	A/G	A/G
	D-Maltose	A/G	A/G	A/G	A/G	A/-	A/G	A/G	A/G
	D-Fructose	A/G	A/G	A/G	A/G	A/-	A/G	A/G	A/G
	D-Mannitol	A/G	A/G	A/G	A/G	A/-	A/G	A/G	A/G
	Dextrose	A/G	A/G	A/G	A/G	A/-	A/G	A/G	A/G
	Myo-inositol	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Lipid hydrolysis		+	+	+	+	+	+	+ weak	- weak
Tentatively identified genus		Unknown	Unknown	Citrobacter	Erwinia	Vibrio	Klebsiella	Aeromonas	Aeromonas
Tentatively identified species		Unknown	Unknown	*Citrobacter youngae*	*Erwinia herbicola*	*Vibrio* sp.	*Klebsiella pneumonia subspecies ozanae*	*Aeromonas cariae*	*Aeromonas cariae*

Key: (+): Positive, (-): Negative, ND: Not done/determine, Y: Yellow, R: Red, +/-:Orange ,NG: No growth, A/G: Acid/Gas, A/-:Acid/No gas, -/-:not acid production/no gas ,NA: No Action , RT: Room Temperature, NF: Non-Fermented ,F: Fermented, HL: Hugh & Leifson, MR: Methyl Red, H_2S: Hydrogen Sulfate, NaCl: Sodium Chloride, NG: No Growth

Table 1. Morphological and biochemical characterization of bacteria isolated from mucus of *Acropora cervicornis* (continued)

CHARACTERISTICS		MD026	MD029a	MD029b	MD030	MD031	APC02	APC05	APC06
Morphology of organisms		Coccobacilli	Short Rod	Coccobacilli	Coccobacilli	Short Rod	Coccobacilli	Short Rod	Short Rod
Colony color		Yellowish	Yellowish	Yellowish	Yellowish	Brownish	Whitish	Creamy yellowish	Colourless
Gram's stain		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Motility		+	+	+	-	-	-	-	+
Catalase activity		+	+	+	-	+	+Weak	-	-
Oxidase activity		+	-	+	-	+	+	+	+
HL media (O/F)		NA	F	F weak	F Weak	NA	ND	ND	ND
VP test		-	-	-	-	-	+	-	-
MR test		-	+	-	-	-	+	+	-
Indole production		-	-	-	-	-	-	-	+
Growth on MacConkey agar		+ NF	-	+ F	+ F	+ NF	+ NF	-	+F
Growth on TCBS agar		+ Green	-	-	-	-	+ Yellow	-	+
Growth on Pseudomonas agar		-	-	-	+ White	+ White	+	+	+
Eosin Methylene Blue agar (EMB)		+ Pink	+ Pink	-	+ Pink	+ Pink	-	-	+ Pink
Growth on Salmonella agar		+ Yellow	-	-	-	-	-	-	+
Simmon citrate utilization		-	+	-	-	-	-	-	-
TSI (Triple Sugar Iron)	Butt (Glucose)	Y	Y	Y	R	Y	Y	R/Y	R/Y
	Gas production	-	+	-	-	+	+	-	-
	H_2S production	+	-	+	-	-	-	-	-
	Slope (Lactose)	R	Y	R	R	R	R/Y	R	R/Y
Casein hydrolysis		+	-	+	-	-	+	-	-
Nitrate reduction		+	+	+	NG	+	+	+	+
Starch Hydrolysis		-	-	-	-	-	+	+	-
Gelatin hydrolysis		-	-	-	-	-	-	-	-
Tween-20 hydrolysis		+	-	+	NG	-	+	-	+
Tween-80 hydrolysis		-	-	-	NG	-	-	-	-
Bioluminescence		-	-	-	-	-	-	-	-

Key: (+): Positive, (-): Negative, ND: Not done/determine, Y: Yellow, R: Red, +/-:Orange ,NG: No growth, A/G: Acid/Gas, A/-:Acid/No gas, -/-:not acid production/no gas ,NA: No Action , RT: Room Temperature, NF: Non-Fermented ,F: Fermented, HL: Hugh & Leifson, MR: Methyl Red, H_2S: Hydrogen Sulfate, NaCl: Sodium Chloride, NG: No Growth

Table 1. Morphological and biochemical characterization of bacteria isolated from mucus of *Acropora cervicornis* (continued)

CHARACTERISTICS		MD026	MD029a	MD029b	MD030	MD031	APC02	APC05	APC06
NaCl Requirement	NA without NaCl	-	+	-	-	+	-	+	-
	3 % NaCl	+	+	+	+	+	+	+	+ Weak
	6 % NaCl	+	+	+	+	+	+	+	+ Weak
	9 % NaCl	-	-	-	+	-	+	-	-
	12 % NaCl	-	-	-	-	-	-	-	-
	15 % NaCl	-	-	-	-	-	-	-	-
	20 % NaCl	-	-	-	-	-	-	-	-
Growth on different temperature	4 ° C	-	-	-	-	-	-	-	-
	RT	+	+	+	+	+	+	+	+
	37 ° C	+	+	+	+	+	+	+	+
	40 ° C	+	+	+	+	+	+	+	+
	50 ° C	-	-	-	-	-	-	-	-
	60 ° C	-	-	-	-	-	-	-	-
Urease activity		-	-	-	-	-	ND	ND	ND
Blood hemolysis		+ά	-γ	+ά	-γ	-γ	-γ	-γ	-γ
Utilization of	Glucose	-/-	A/G	-/-	-/-	-/-	A/-	A/-	A/-
	Lactose	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	Galactose	-/-	A/G	-/-	/-	A/-	-/-	A/-	A/-
	Sucrose	-/-	A/G	-/-	-/-	-/-	A/-	-/-	-/-
	D-Arabinose	-/-	A/G	-/-	-/-	-/-	-/-	-/-	-/-
	D-Maltose	-/-	A/G	-/-	-/-	-/-	A/-	-/-	A/-
	D-Fructose	-/-	A/G	-/-	-/-	-/-	A/-	A/-	A/-
	D-Mannitol	-/-	A/-	-/-	-/-	-/-	A/-	A/-	A/-
	Dextrose	-/-	A/G	-/-	-/-	-/-	A/-	-/-	A/-
	Myo-inositol	-/-	A/-	-/-	-/-	-/-	-/-	-/-	-/-
Lipid hydrolysis		+	+	+	+	+	+ weak	+	+
Tentatively identified genus		Alteromonas	Serratia	Alteromonas	Moraxella	Brucella	Photobacterium	Flavobacterium	Unknown
Tentatively identified species		*Alteromonas putrefaciens*	*Serratia* sp.	*Alteromonas* sp.	*Moraxella* sp.	*Brucella* sp.	*Photobacterium* sp.	*Flavobacterium* sp.	Unknown

Key: (+): Positive, (-): Negative, ND: Not done/determine, Y: Yellow, R: Red, +/-:Orange ,NG: No growth, A/G: Acid/Gas, A/-:Acid/No gas, -/-:not acid production/no gas ,NA: No Action , RT: Room Temperature, NF: Non-Fermented ,F: Fermented, HL: Hugh & Leifson, MR: Methyl Red, H_2S: Hydrogen Sulfate, NaCl: Sodium Chloride, NG: No Growth

Table 1. Morphological and biochemical characterization of bacteria isolated from mucus of *Acropora cervicornis* (continued)

CHARACTERISTICS		APC08	APC015	APC018	APC019	APC020
Morphology of organisms		Short Rod	Short Rod	Short Rod	Short Rod	Short Rod
Colony color		Creamy white	Whitish	Creamy	Whitish	Creamy white
Gram's stain		Negative	Negative	Negative	Negative	Negative
Motility		-	-	-	-	-
Catalase activity		-	+	+ Weak	+	+
Oxidase activity		+	-	-	-	-
HL media (O/F)		ND	ND	ND	NA	ND
VP test		-	-	-	-	+
MR test		-	+/-	+	-	+
Indole production		-	-	-	-	-
Growth on MacConkey agar		+ NF	-	+ NF	-	+ NF
Growth on TCBS agar		+ Green	-	+ Yellow	-	+ Yellow
Growth on Pseudomonas agar		-	+	+	+	+
Eosin Methylene Blue agar (EMB)		+ Pink	-	-	-	+
Growth on Salmonella agar		-	+	-	+	+ Pink
Simmon citrate utilization		-	-	-	-	-
TSI (Triple Sugar Iron)	Butt (Glucose)	Y	R	R	Y	R/Y
	Gas production	+	-	-	+	+
	H_2S production	-	-	-	-	-
	Slope (Lactose)	R	R	R	Y	Y
Casein hydrolysis		-	+	-	+	-
Nitrate reduction		+	+	+	+	+
Starch Hydrolysis		-	+	+	+	+
Gelatin hydrolysis		-	-	-	-	-
Tween-20 hydrolysis		+	+	+	-	-
Tween-80 hydrolysis		-	-	NG	+	+
Bioluminescence		-	-	-	-	-

Key: (+): Positive, (-): Negative, ND: Not done/determine, Y: Yellow, R: Red, +/-:Orange ,NG: No growth, A/G: Acid/Gas, A/-:Acid/No gas, -/-:not acid production/no gas ,NA: No Action , RT: Room Temperature, NF: Non-Fermented ,F: Fermented, HL: Hugh & Leifson, MR: Methyl Red, H_2S: Hydrogen Sulfate. NaCl: Sodium Chloride. NG: No Growth

Table 1. Morphological and biochemical characterization of bacteria isolated from mucus of *Acropora cervicornis* (continued)

CHARACTERISTICS		APC08	APC015	APC018	APC019	APC020
NaCl Requirement	NA without NaCl	-	+	+		-
	3 % NaCl	+	+	+	+	+
	6 % NaCl	-	-	+ Weak	+	+
	9 % NaCl	-	-	+ Weak	+	+
	12 % NaCl	-	-	-	-	-
	15 % NaCl	-	-	-	-	-
	20 % NaCl	-	-	-	-	-
Growth on different temperature	4 ° C	-	-	-	-	-
	RT	+	+	+	+	+
	37 ° C	+	+	+	+	+
	40 ° C	+	+	+	+	+
	50 ° C	-	-	+	-	-
	60 ° C	-	-	-	-	-
Urease activity		ND	ND	ND	ND	ND
Blood hemolysis		-γ	+β	-γ	-γ	-γ
Utilization of	Glucose	A/G	A/-	-/-	A/-	A/-
	Lactose	-/-	-/-	-/-	-/-	-/-
	Galactose	A/G	-/-	-/-	A/-	-/-
	Sucrose	-/-	A/-	-/-	A/-	A/-
	D-Arabinose	-/-	-/-	-/-	-/-	-/-
	D-Maltose	A/G	A/-	-/-	A/-	A/-
	D-Fructose	A/G	A/-	A/-	A/-	A/-
	D-Mannitol	A/G	A/-	A/-	A/-	A/-
	Dextrose	-/-	-/-	-/-	A/-	A/-
	Myo-inositol	-/-	-/-	-/-	-/-	-/-
Lipid hydrolysis		+weak	+weak	-	-	+weak
Tentatively identified genus		Vibrio	Yersinia	Vibrio	Acinetobacter	Yersinia
Tentatively identified species		*Vibrio* sp.	*Yersinia bercerieri*	*Vibrio netschnikonii*	*Acinetobacter* sp.	*Yersinia enterocolitica*

Key: (+): Positive, (-): Negative, ND: Not done/determine, Y: Yellow, R: Red, +/-:Orange ,NG: No growth, A/G: Acid/Gas, A/-:Acid/No gas, -/-:not acid production/no gas ,NA: No Action , RT: Room Temperature, NF: Non-Fermented ,F: Fermented, HL: Hugh & Leifson, MR: Methyl Red, H_2S: Hydrogen Sulfate. NaCl: Sodium Chloride. NG: No Growth

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REFERENCES

1. Paulay G. (1997). Diversity and distribution of reef organisms. In: *Life and Death of Coral Reef* (ed. C. Birkeland). Chapman and Hall, New York. 298 - 353.
2. Forest R., Seguritan V., Azam F. and Knowlton N. (2002). Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series* **243**: 1 – 10.
3. Wilkinson E. B. C. (2003). *Status of Coral Reefs of the World: 2002.* Australian Institute of Marine Science, Cape Ferguson, Townsville, Queensland, Australia.
4. Ducklow H. W. and Mitchell R. (1979). Bacterial populations and adaptations in the mucus layers on living corals. *Limnology of Oceanography* **24**: 715 – 725.
5. Shashar N., Cohen Y. and Loya N. (1994). Nitrogen fixation in stony corals: Evidence for coral-microbial interactions. *Marine Ecological Progress Series* **111** (3): 259 - 264.
6. Rohwer F., Breithart M., Jara J., Azam F. and Knowlton N. (2001). Diversity of bacteria associated with the Caribbean coral *Montastraea franksi. Coral Reefs* **20**: 85 - 91.
7. Borneman, E. (1998). Bacteria and Coral: Good Or Bad? (online). http://www.reefs.org/library/talklog/e_bornemman_051098.html (Accessed 13th June 2005).
8. Salvo L. H. (1969). Isolation of bacteria from the corallum of *Porites lobata* (Dana) and its possible significance. *American Zoology* **9**: 735 - 740.
9. Vacelet E. and Thomassin B. (1991). Microbial utilization of coral mucus in long term in situ incubation over a coral reef. *Hydrobiologia* **211**: 19 – 32.
10. Richardson L.L. (1998). Coral diseases: what is really known? *Trends Ecology Evolutionary* **13**: 438 – 443.
11. Harvell C.D., Kim K., Burkholder J.M., Colwell R.R., Epstein P. R., Grimes D. J., Hofmann E. E., Lipp E. K., Osterhaus A.D., Overstreet R. M., Porter J. W., Smith G. W. and Vasta G.R. (1999). Emerging marine diseases — climate links and anthropogenic factors. *Science* **285**: 1505 - 1510
12. Giovannoni S. and Rappe M. (2000). Evolution, diversity, and molecular ecology of marine prokaryotes. In: *Microbial Ecology of the Oceans* (ed. D.L. Kirchman) Wiley-Liss, New York, p 47 – 84.
13. Rohwer F., Seguritan V., Azam F., and Knowlton N. (2002). Diversity and distribution of coral-associated bacteria. *Inter-research* **243**: 1 - 10.
14. Knowlton N., and Rohwer F. (2003). Multispecies Microbial Mutualisms on Coral Reefs: The Host as a Habitat, *The American Naturalist* **162**: S51-S62.
15. Oppenheimer C.H. and Zobell C.E. (1952). The growth and viability of sixty-three species of marine bacteria are influenced by hydrostatic pressure. *Journal of Marine Research* **11**: 10 – 18.
16. Buck J.D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Journal of Applied Environmental Microbiology* **44**: 992 – 993.
17. Okutani K. (1985). Taxonomical Studies of Polysaccharide Producing Bacterium From Sea Cecumber.Stihopus Japonicus (Selenka). *Technical Bulletin of Faculty of Agriculture, Kagawa University* **36** (2): 76: 135 - 140.
18. Farrow J.A.E., Wallbanks S, and Collins M.D. (1994). Phylogenetic interrelationship of round-spore-forming bacilli containing cell walls based on lysine and the non-spore-forming genera *Caryophanon, Exiguobacterium, Kurthia, and Planococcus. International Journal of Systematic Bacteriology* **44**: 74 – 82.
19. Ivanova E.P., Nicolau D.V., Yumoto N., Taguchi T., Okamoto K., Tatsu Y. and Yoshikawa S. (1998). Impact of the conditions of cultivation and adsorption on antimicrobial activity of marine bacteria. *Marine Biology* **130**: 545 – 551.
20. MacLeod R.A. (1965). The question of the existence of specific marine bacteria. *Journal of Bacteriology Review* **29**: 9 - 23.

21. MacLeod R.A. (1968). On the role of inorganic ions in the physiology of marine bacteria. In: *Advances in Microbiology of the Sea* (ed. M.R Droop and E. J. F Wood). London, New York: Academic Press. 95 - 126.
22. Taylor C.B. and Lochhead A.G. (1938). Qualitative studies of soil microorganisms. II. A survey on the bacterial flora of soils differing in fertility. *Can. J. Res., Sec. C.* **16**: 162 - 173.
23. Kelly K. and Chistoserdov A. (2001). Phylogenetic analysis of the succession of bacterial communities in the Great South Bay (Long Island). *FEMS Microbiology of Ecology* **35**: 85 - 95.
24. Ritchie K.B. and Smith G.W. (1998). Type II white-band disease. *Review Biology Tropical* **46**: 199 - 203.
25. Michael A.P., Michael J.S. and Loretta B. (1989). Isolation of bioactive actinomycetes from marine sediments using rifampicin, *Applied Microbiology Biotechnology* **31**: 609 - 612.
26. Hutson R.A., Thompson D.E. and Collins M.D. (1993). Genetic interrelationships of saccharolytic Clostridium botulinum types B, E and F and related clostridia as revealed by small-subunit rRNA gene sequences. *FEMS Microbiology Letter Marine*. **15**:108 (1): 103 - 110.