Bacteria in Sediments in a Seagrass Bed in Kauswagan, Lanao del Norte (Mindanao, Philippines)

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Abstract

Benthic bacteria found in the muddy sediments of a seagrass bed in the coastal waters of Kauswagan, Lanao del Norte were studied. Sediment samples were taken during low tide with the use of a modified core out of a plastic syringe. Three series of sample dilution were made and aliquots were prepared, isolated and cultured.

Results of the morphological, cultural and physiological tests showed six bacteria belonging to five (5) genera, namely: ***Vibrio parahaemolyticus, V. cholerae, Pseudomonas, Bacillus, Salmonella*** *and* ***Shigella.***

No conclusive evidence could be said on the origin of the bacteria nor of the number of species found due to limited method available for identification. The bacterial flora may show the type of sediment and heterotrophic activities occurring in the seagrass sediments.

Introduction

Seagrass communities are considered as among the most productive in the marine environment producing between 500 and 1000 g C/m^2 y (McRoy, 1970; Mann, 1972). These thrive extensively in shallow coastal waters and estuaries in tropical, subtropical, and temperate regions of the world (Ferguson *et al.*, 1981). They act as sediment stabilizers, as sediment traps, substrate for epiphytes and epizoa and are highly sufficient in nutrient strippings from water as well as sediment (e.g. den Hartog, 1977; Dawes, 1981). Seagrass beds serve as nursery ground for many species of organisms that spend their adult lives in other areas (Kikuchi & Peres, 1977; Nybakken, 1982).

In these environment, the microorganisms including algae, bacteria and fungi make up a large proportion of the total biomass. Marine bacteria carry out a number of significant biological functions in the marine environment especially in the anaerobic processes in the sediments. Some of the

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bacterial activities include converting and translocating minerals, producing and decomposing seagrass tissues and other organic matter forming part of the detritus which are available to other marine consumers (Kikuchi & Peres, 1977; Meyer-Reil, 1984a; Rheinheimer, 1984). Bacteria which are believed to be nitrogen-fixers are abundant in the seagrass beds (Wicks, 1980).

Although marine bacteria are important components of the marine ecosystem, these are less studied in the Philippines and in Iligan Bay, in particular (Lacuna, 1986; Limbaco, 1986; Tan, 1988). To fill in the gap in our knowledge on marine bacteria and to serve as baseline data on the nutrient recycling in the seagrass beds, this study was done.

Methodology

The area of study in Iligan Bay is situated in Kauswagan, Lanao del Norte which has a large intertidal flat ca. 500 m long from the shoreline. The first 60 m is characterized as muddy substrate and is inhabited by the luxuriant growth of seagrasses, predominantly *Enhalus acoroides*, which are fully submerged in water even at low tide (Apao & Camarao, 1983).

Sediment samples were carried out during low tide at five (5) sampling substations with one sample each made in the seagrass bed. A modified core made of ordinary 10 ml- disposable syringe (6.9 cm long and 1.2 cm diameter) cut at one end was used to collect sediment samples by pushing deep down into the muddy sediment at a depth equal to the length of the core. The core was withdrawn with the lower end supported to prevent removal of sediment samples. Samples were carefully transferred to sterile vials and stoppered.

A gram of sediment sample was suspended in a 9-ml sterile seawater blank and serial dilutions were made (1:10; 1: 100; 1: 1000). Aliquots from each diluted sample were placed in sterile dishes with nutrient agar (NA) and were incubated for 24 hours. Well isolated colonies formed in the dishes were collected, the selection of which were based on the differences of colony, form and shape. Further culture in NA plates and slants were made. Side culture study was also conducted using selective media such as Thiosulfate Citrate-Bile-Sucrose (TCBS) and Eosin-Methylene Blue (EMB) Agar.

Isolates selected from colonies formed were subjected to morphological, cultural and physiological tests using standard methods (Buchanan & Gibbous, 1974). Morphology refers to Gram-, capsule- , and spore-staining reactions. In addition to the size, arrangement of vegetative cells as well as the presence of capsule and spore were noted. The presence or absence of motility was observed using the hanging-drop method (Raymundo *et al.*, 1991). Growth behaviour of bacteria in culture plate and slants were observed for the cultural tests.

Physiological tests were done for H_2S production using Triple Sugar Iron Agar (TSIA) medium; glucose and sucrose fermentation with Methyl Red-Vogues Proskauer (MR-VP) test; catalase test using Nutrient Agar; oxidation/fermentation test using Hugh & Leifson media; Citric Acid utilization with the use of Simon's Citrate Agar and nitrate reduction using nitrate agar (Buchanan & Gibbous 1974; Merck, 1982; Raymundo *et al.*, 1991).

Inoculated media were incubated at 37°C unless otherwise indicated.

Results and Discussion

A total of eighteen (18) distinct colonies from 1:100 and 1:1000 dilutions were observed, selected and isolated from the sediment samples. Of these eighteen colonies, ten (10) were identified from the selective media while eight were selected from the biochemical tests. Tables 1 and 2 show the morphological, cultural and physiological characteristics of the isolates and their genus level.

Table 1. Morphological, cultural and physiological characteristics of bacterial isolates in nutrient agar (NA) and various media for biochemical tests. These are used as bases for identification of the bacteria. Sediment samples were taken in a seagrass bed in Iligan Bay, Kauswagan, Lanao del Norte.

Characteristics :	Bacterial Isolates							
	2	6	7	14	15	16	17	18
Morphological								
size (mm)	1.8	2.5	2.0	2.0	2.0	3.0	2.0	2.0
motility	+	-	+	+	+	+	-	-
gram reaction	-	+	-	-	-	-	-	-
capsule	-	-	-	-	-	+	-	-
spore	-	+	-	-	-	-	-	-
arrangement	s	s	s	s	s	s	s	s
shape	r	r	r	r	r	r	r	r
Cultural								
NA slants								
form	B	B	A	A	A	A	A	F

Table 1 continued

NA plates								
form	F	F	F	F	F	F	F	F
margin	F	U	E	E	E	E	F	F
elevation	FL	FL	FL	FL	FL	FL	FL	FL
Physiological								
Triple Sugar Iron Agar								
glucose ferm.	+	+	-	-	-	+	+	+
sucrose ferm.	-	-	-	-	-	-	-	-
gas production	-	-	-	-	-	+	-	-
H_2S prod.	-	+	-	-	-	+	-	-
Methyl Red Vogues Proskauer								
Methyl Red	-	+	-	-	-	+	+	+
Vogues Proskauer	-	-	-	-	-	-	-	-
Simon's Citrate	-	+	-	-	-	+	+	-
Nitrate Broth								
Reduction of nitrate to nitrate	+	+	+	+	+	+	+	+
Hugh & Leifson								
oxidative	+	-	+	+	+	+	-	-
fermentative	-	+	-	-	-	-	+	+
Enzymes								
catalase	+	+	+	+	+	+	+	+
GENUS	Ps	Ba	Ps	Ps	Ps	Sa	Sh	Sh

Legend:

+ positive/present
- negative/absent
s single, R-rod
E - entire
FL- flat

A - arborescent
B - beaded
F - filiform
U - undulate

Ba - Bacillus
Ps - Pseudemonas
Sa - Salmnella
Sh - Shigella

Vibrio (*PV. parahaemolyticus, V. cholerae*) were identified from the sediments. *Pseudomonas* was found in three subsampling stations, *Salmonella* and *Shigella* in two substations and *Bacillus* in one substation (Table 3).

Table 2. Cultural characteristics of bacterial isolates grown on selected media, Thiosulfate Citràte Bile Sucrose Agar (TCBS) and Eosin Methylene Blue (EMB). Sediment samples were taken from a seagrass bed in Kauswagan, Lanao Norte.

Selective Media	Description of colony Form	Size (mm)	Color	Species/Genus
TCBS				
	flat	2-3	yellow	*Vibrio cholerae*
	flat	< 1	blue-green	*V. Parahaemolyticus*
EMB				
	flat	2	translucent	*Salmonella*
	flat	2	amber	*Shigella*

Table 3. Bacteria isolated from sediment samples taken at Kauswagan, Lano del Norte in five substations. (+ present; - absent).

Bacteria	Substation 1	2	3	4	5
1. Bacillus	-	+	-	-	-
2. Pseudonymous	+	+	-	-	+
3. V. cholerae	+	+	+	-	-
4. V. parahaemolytics	+	+	+	-	+
5. Salmonella	-	-	-	-	+
6. Shigella	-	-	-	-	+

Most of the isolates from the marine sediment were Gram-negative (G-) with *Bacillus* as the lone Gram-positive (G+) representative conforming to the general observations that 95 % of marine bacteria are G- (Rheinheimer 1984; Dawes, 1981). All of the identified bacteria were rod-shaped and small in size, the results of which are similar to other studies (Rheinheimer, 1983).

The type of bacteria observed in the sediments serves as basis for the characterization of sediment type in the muddy substrate in a seagrass bed. The sediment in the sampling sub- station exuded noxious smell characteristic of sulfur when disturbed especially during collection (pers. observations). This may imply that the sediment is anoxic as sediments of most shallow-water localities especially those of seagrass beds become chemically reducing a few millimeters below the surface and show very steep gradient in oxygen concentration (Fenchel, 1977). In addition, *Salmonella* and *Bacillus* from the sediment produced hydrogen sulfide which may indicate the capability of utilizing sulfur compounds from the sediment. Hydrogen sulfide tends to accumulate in the sewage-loaded ponds and harbour basins. Sulfur bacteria are common in the sediments (Zobell, 1946) and the main producers of hydrogen sulfide in the marine environments are anaerobic species of *Desulfovibrio* (Dawes, 1981) which were not observed in the study area.

All bacteria isolated from the sediment were positive of the catalase test proving the generalization that benthic bacteria are capable of preventing the effect of noxious substances that will be formed by oxygen metabolism (Stainer, 1979).

Faecal pollution of marine waters is suspected as source of *Salmonella* in the sediment in the seagrass bed which is situated directly at the shore lined with residential houses whose sewage were loaded more or less directly into the waters (pers. observations). These organisms might be considered transient in the sediment and not autochthonous.

Vibrio is reported to be one of those bacterial pathogens which cause infection in marine plants such as algae and marine animals. Similar to *Cytophaga* (Reichardt *et al.*, 1983), *Vibrio* are able to digest chitin.

The presence of these bacteria which are commonly of human faecal origin and are usually pathogenic may raise alarm as to the safety and sanitation of the marine waters especially for bathers. It may also raise questions on the safety of seafoods such as sea urchins and shells collected from the area. Since no quantification of bacteria was made, it could not be said that the area is beyond the sanitation standard set for marine waters.

Between the overlying waters and sediments, there is a considerable cycle of nutrients and substances. During the death of an algae, there is released of great amount of organic substances during fragmentation which is later acted upon by bacteria. The organic matter is converted by microorganisms into compounds with smaller energy content and finally under appropriate conditions, into the original mineral substances. In the case of marine plants, the limiting nutrients are recycled and are utilized for growth and other metabolic processes.

Preliminary studies on the identification of bacteria in various sediments in Iligan Bay yielded species not present in this study such as *Spirillum, Proteus, Photobacteria,* and *Flavobacterium* (Tan, 1986). The differences maybe attributed to the sediment types studied (Rheinheimer, 1983; Reichardt *et al.*, 1983). Muddy substrates are reported to have greater bacterial number than sandy sediments and generally, there is an increase in bacterial population in the presence of benthic vegetation compared to bare sediments (Mow-Robinson & Rheinheimer, 1985).

Bacterial counts differ between sediment types ranging from 1 x 10^8 - 5.07 x 10^8 cells per g dry weight sediment (Meyer-Reil, 1984a). This difference in the bacterial biomass maybe correlated with the organic matter content of the sediment which serve as substrate for bacterial action (Meyer-Reil 1984b).

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