

Pathogenic Bacteria in Corals from Veracruz Reef System National Park

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Abstract: The Veracruz Reef System National Park (PNSAV) is situated in front of Veracruz-Boca del Río and Antón Lizardo conurbation with approximately 65,516 hectares. In 1992 this reef system was declared a Natural Protected Area and in 2006 it became Biosphere reserve. Despite this recognition, the PNSAV is exposed to anthropogenic effects such as river discharge, port traffic, tourism, and waste discharges; all this causes sediment and bacteria, which affect corals. Studies conducted by PNSAV show the presence of diseases and syndromes in scleractinian corals but do not denote the causal agent of the disease. For this reason, the present study aimed to make the diagnosis of bacteria causing damages to the scleractinian coral and its relation to discharges from areas of influence of PNSAV. 12 fixed 80 meter transects were established and coral samples were collected from the genera: *Colpophyllia*, *Montastraea*, *Porites* and *Siderastrea*. The lesions identified as criteria for the collection were bands, marks and spots on the body of the coral. The sample was run in duplicate, both the affected and the visually healthy. A microbiological analysis of wastewater from point sources of pollution was performed. The coral and water samples were analyzed by simple PCR in laboratory of the Instituto Tecnológico de Boca del Río in Veracruz (ITBOCA). Primers of pathogenic bacteria include species of the genera *Oscillatoria*, *Vibrio*, *Serratia* y *Aurantimonas*. There was a positive presence of *Vibrio* bacteria with an 87% in the water samples and a 27.1% in coral tissue. The bacteria *Serratia marcescens* was only present in water samples and the *Aurantimona coralicida* in diseased coral tissue. It was also noted that there is a relationship in bacteria in the positive presence of 3-point sources of pollution and coral tissue.

Keywords: Pathogenic bacteria, Corals, PCR.

I. Introduction

In recent years it has been considered that coral reefs worldwide are threatened with extinction, this as a result of climate change and anthropogenic pollution, which generates a large-scale mortality and a difficult long-time regeneration [1] [2] [3]. The rapid growth of human population on coastal margins of the surrounding reefs has caused physical, chemical and biological changes in these systems [4]. Point sources of pollution are generally discharges through pipes and channels, which are taken to riverbeds, coastal waters and other water bodies. All these sources provide bacteria causing adverse effects difficult to control [5] [6].

Bacterial communities are known to be extremely important in many ecosystems. In coral reef systems, the bacteria are sensitive to environmental or anthropogenic changes and even after these changes occur they continue to cause effects over long periods of time [7].

This pathogenic bacteria causes degradation of coral tissue and during that process it quickly colonizes the coral structure altering significantly the microbial community (Holobiontes), and its interaction with the host [8]. The injuries caused by bacterial infection in the coral are identified as bands, irregular spots and dots.

The National Park Veracruz Reef System (PNSAV) is exposed to local effects such as discharges from the Jamapa River with a discharge volume of 670 million m³ per year, it also has the influence of the Papaloapan River with a discharge volume of 20,000 million m³ a year [9], and to the north is the influence of La Antigua River. The PNSAV is influenced by the port traffic, which admitted between 1505 and 1809 ships a year [10]. Added to this, there are multiple impact factors such as the flow of municipal, industrial and agricultural discharges, tourism including scuba diving, boat trips and visits to some of the beaches and shallow areas of the park, involving among other vessels anchoring, groundings, and oil spills [11].

The river discharges are rich in nutrients and sediment. These characteristics cause adverse affectations for coral reefs since these systems prefer oligotrophic conditions and clear water [12].

There is little information concerning the identification of pathogenic bacteria that cause damage to the health of corals in the Gulf of México. Understanding the need of information, this study generates the first diagnosis of pathogenic bacteria in corals of PNSAV.

II. Materials and Method

2.1 Study area

This study was conducted at the Veracruz Reef System National Park (PNSAV), it is located between the coordinates 19°00'00" – 19°16'00" N and 95°45'00" – 96°12'00" W, in the central portion of the state of Veracruz. The PNSAV extension is 65,516 hectares (Fig. 1).

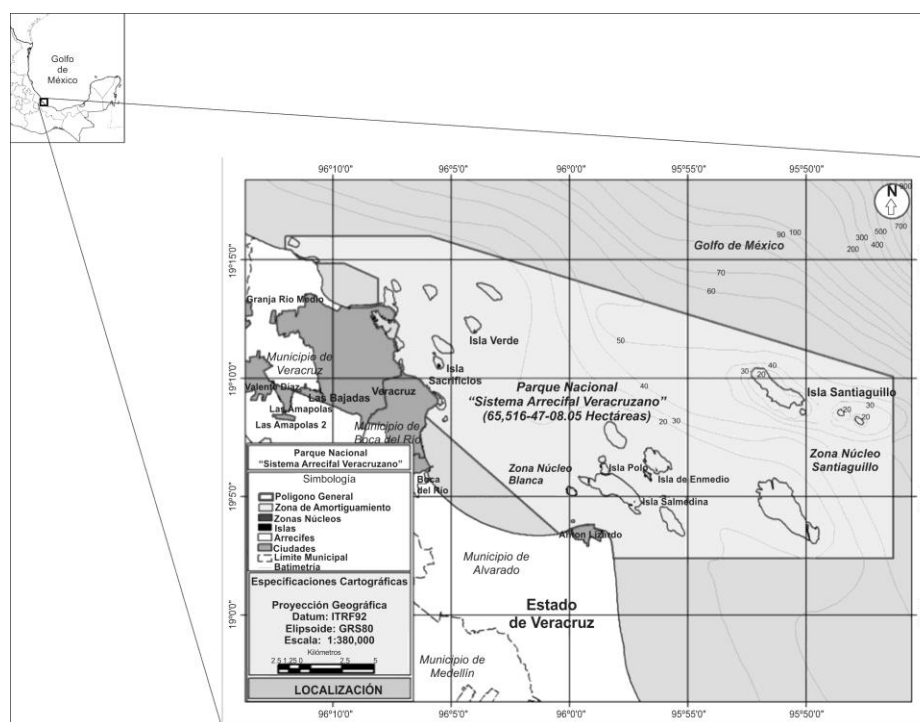


Figure 1. Schematic location of PNSAV.

2.2 Sample collection and process

2.2.1 Point sources of pollution (PSP)

Ten point sources of pollution were identified and selected; they are located throughout the metropolitan area of Veracruz-Boca del Río, Alvarado and Antón Lizardo. The sites selected were: Papaloapan River, Giote Stream, Jamapa River, Villa Rica Sump, América's Calzada, Simón Bolívar Collector, Yacht Club, Port Area, Shipyards and an open sky channel The Cable. There were obtained 10 samples with replicates at each sampling site. The water samples were placed in sterile polyethylene bags and transported in coolers at an approximate temperature of 4°C for preservation until analysis in a maximum of 5 hours in the Research Laboratory and Aquatic Resources of the Instituto Tecnológico de Boca del Río (ITBOCA).

2.2.2 Corals

The coral tissue sampling was performed by setting 12 fixed 80 x 1m transects with a parallel pattern to the coast (northeast-southwest). A discriminatory table was performed as proposed criteria for the selection of coral species based on greater distribution and occurrence of diseases that resulted in the following species: *Colpophyllia natans*, *Montastraea cavernosa*, *Montastraea faveolata*, *Porites astreoides* and *Siderastrea siderea*.

The sampling consisted on removing an approximately 2cm³ piece of coral tissue; a chisel and a hammer were used as support tools for the extraction of the tissue sample. The tissue sample was extracted in

duplicate, both visually impaired and healthy part. These were collected separately in sterile bags and stored at an approximate temperature of 4°C for storage until analysis in a maximum period of 5 hours at the laboratory of ITBOCA.

Water samples and corals were processed for DNA extraction using PROMEGA Corp. The molecular biology test that was performed was simple PCR and the primers used were pathogenic bacteria, as shown in the following table (Table 1).

2.2.3 Statistical analysis

The test of Cochran, a non-parametric statistical method was used by means of a randomized complete block design. The data generated were analyzed by the Statistica software V.10.

Bacteria	Primer	Sequence	Base pairs	References
Género Oscillatoria	Antx-f Antx-r	CGCAAATCGATGCTCACTTA CCACTGGCTCCATCTTGATT	650	[13]
<i>Aurantimonas coralicida</i>	Ac-995F UB-1492R	TCG ACG GTA TCC GGA GAC GGA T TAC GGY TAC CTT GTT ACG ACT T	500	[14]
<i>Vibrio complex</i>	HA-F HA-R	CATGAGGTCAGCCACGGTTTTACTGAGCAG CGCGCGGTTAAACACGCCACTCGAATGGTGAAC	225	[15]
<i>Serratia marcescens</i>	Sm-456F UB-1492R	GGT GAG CTT AAT ACG TTC ATC A TAC GGY TAC CTT GTT ACG ACT T	1040	[14]
<i>Vibrio coralliilyticus</i>	Vc-76F Vc-1019R	GTT RTC TGA ACC TTC GGG GAA CG CTG TCT CCA GTC TCT TCT GAG G	940	[14]
<i>Vibrio shilonii</i>	Vs-457F Vs-1023R	GGT ACG TTA ATA GCG TGC TCG ACC TGC GTC TCC GCT GGC	570	[14]

Table 1. Pathogenic bacteria and primers reported by different authors for corals.

III. Results

3.1 Pathogenic bacteria on PSP

Pathogenic bacteria on PSP (n=10) shows the presence of 85% of *Vibrio complex* (Fig. 2), 45% of *Vibrio coralliilyticus*, 45% of *Vibrio shilonii*, 60% of *Serratia marcescens* and absence of *Aurantimonas coralicida* and *Oscillatoria* genus.

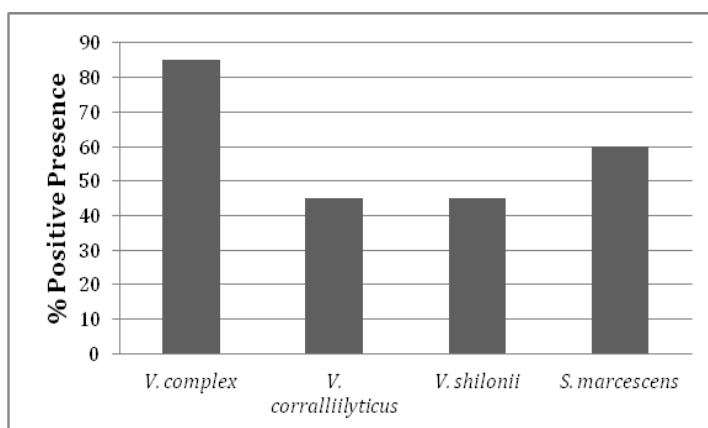


Figure 2. Positive presence of pathogenic bacteria in the point sources of pollution (PSP) of PNSAV.

Presence of pathogenic bacteria in the PSP of PNSAV showed to *V. complex* present in each sample point. *Aurantimonas coralicida* and the *Oscillatoria* genus were absent (Table 2).

Table 2. Presence of pathogenic bacteria in the PSP of PNSAV. (Vcom) *Vibrio complex*, (Vc)*Vibrio coralliilyticus*, (Vs)*Vibrio shilonii*, (Sm)*Serrata marcescens*, (Ac)*Aurantimonas coralicida*, (Os)*Oscillatoria*; (+) positive, (-) negative.

Point sources of pollution	V. com	Vc	Vs	Sm	Ac	Os
River Papaloapan	+	+	+	+	-	-
Stream Giote	+	+	+	+	-	-
River Jamapa	+	+	+	+	-	-
Lift station Villa Rica	+	+	-	+	-	-
Calzada de las Américas	+	-	-	-	-	-
Collector Simón Bolívar	+	-	-	-	-	-
Yacht club, Veracruz	+	+	+	+	-	-
Port area, Veracruz	+	+	+	+	-	-
Shipyard, Veracruz	+	-	-	-	-	-
Waterway El Cable	+	+	-	+	-	-

3.2 Corals

The pathogenic bacteria present in coral tissue (n=92) showed 42.3% for *Vibrio complex*, 27.1% for *Vibrio coralliilyticus*, 11% for *Vibrio shilonii*, 11% for *Aurantimonas coralicida* and absence of *Serratia marcescens* and *Oscillatoria* genus (Fig. 3).

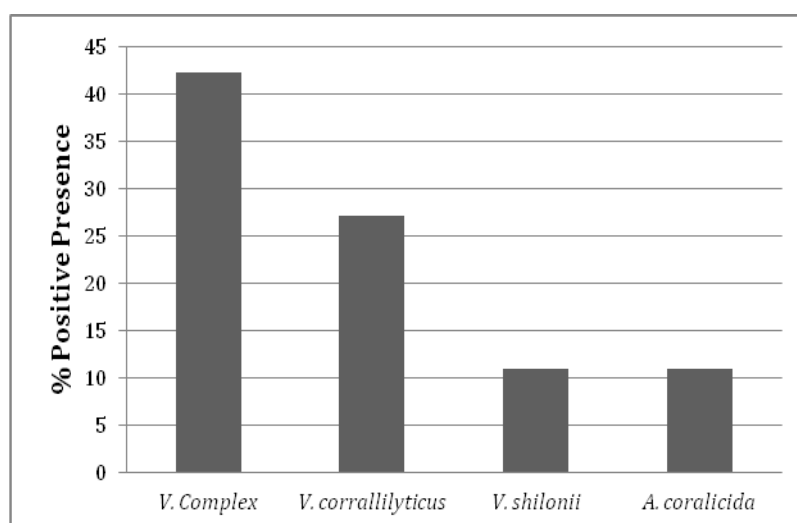


Figure 3. Positive presence of pathogenic bacteria *C. natans*, *M. Cavernosa*, *M. Faveloata*, *P. Astreoides*, *S. Siderea* identified in coral tissue in the PNSAV.

3.3 Relationship of pathogenic bacteria found in the PSP and the PNSAV.

The relationship between the two can be seen in the following comparison table, which was made with the results of the identification of pathogenic bacteria in the PSP and coral tissue (Table 4).

Table 4. Pathogenic bacteria such as (Vcom) *Vibrio complex*, (Vc) *Vibrio Coralliilyticus*, (Vs) *Vibrio Shilonii*, (Sm) *Serratia marcescens*, (Ac) *Aurantimonas coralicida*, (Os) *Oscillatoria* present in the PCP and coral tissue (*C. natans*, *M. Cavernosa*, *M. Faveolata*, *P. Astreoides*, *S. Siderea*). Superscripts ⁽¹⁾⁽²⁾⁽³⁾⁽⁴⁾ refer to the coral species present in each of the reefs of the corresponding row.

Point sources of pollution	Detected bacteria	Near reef	Coral species	Etiological characteristics	Detected bacteria	Bacteria in common
Papaloapan River	Vcom, Vc, Vs, Sm	Cabezo norte ¹ Cabezo sur ² Santiaguillo ³	<i>Siderastrea siderea</i> ^{1,2}	Gray spots	Vcom, Vc	Vcom, Vc
			<i>Siderastrea siderea</i> ²	Yellow spots	Vcom, Vc, Vs	
			<i>Colpophyllia natans</i> ^{1,2}	Bleaching	Vcom, Vc, Ac	
			<i>Porites astreoides</i> ³	Yellow spots	Vcom, Vc, Vs	
Giote Stream	Vcom, Vc, Vs, Sm	Blanca ¹ Giote ² Sargazo ³ La palma ⁴	<i>Siderastrea siderea</i> ¹	White band	Vcom, Vs	Vcom, Vc, Vs
			<i>Siderastrea siderea</i> ²	White spots	Vcom, Ac	
			<i>Siderastrea siderea</i> ³	Gray spots	Vcom, Vs	
			<i>Siderastrea siderea</i> ⁴	Black band	Vcom, Vc	
			<i>Colpophyllia natans</i> ³	Bleaching	Ac	
			<i>Colpophyllia natans</i> ⁴	White band	Vcom, Vc	
			<i>Montastraea faveolata</i> ²	Yellow spots	Vcom, Vc	
			<i>Montastraea cavernosa</i> ¹	White band	Vcom, Vc, Ac	
Jamapa River	Vcom, Vc, Vs, Sm	Verde ¹ Bajo mersey ² Blanca ³ Giote ⁴	<i>Siderastrea siderea</i> ¹	Dark band	Vcom, Vc, Vs	Vcom, Vc, Vs
			<i>Siderastrea siderea</i> ³	White band	Vcom, Vs	
			<i>Siderastrea siderea</i> ⁴	White spots	Vcom, Ac	
			<i>Porites astreoides</i> ²	Yellow spots	Vcom, Vs	
			<i>Colpophyllia natans</i> ¹	Bleaching	Ac	
			<i>Montastraea cavernosa</i> ²	Dark band	Vcom	
			<i>Montastraea cavernosa</i> ³	White band	Vcom, Vc, Ac	
			<i>Montastraea faveolata</i> ⁴	Yellow spots	Vcom, Vc	
Villa Rica Sump	Vcom, Vc, Sm	Verde ¹ Bajo mersey ²	<i>Siderastrea siderea</i> ¹	Dark band	Vcom, Vc, Vs	Vcom, Vc
			<i>Porites astreoides</i> ²	Yellow spots	Vcom, Vs	
			<i>Colpophyllia natans</i> ¹	Bleaching	Ac	
			<i>Montastraea cavernosa</i> ²	Dark band	Vc	
América's Calzada	Vcom	Verde ¹ Bajo mersey ²	<i>Siderastrea siderea</i> ¹	Dark band	Vcom, Vc, Vs	Vcom
			<i>Porites astreoides</i> ²	Yellow spots	Vcom, Vs	
			<i>Colpophyllia natans</i> ¹	Bleaching	Ac	
			<i>Montastraea cavernosa</i> ²	Dark band	Vc	
Simón Bolívar Collector	Vcom	Bajo mersey ¹	<i>Porites astreoides</i> ¹	Yellow spots	Vcom, Vs	Vcom
			<i>Montastraea cavernosa</i> ¹	Dark band	Vcom	
Yacht Club, Veracruz	Vcom, Vc, Vs, Sm	Blanquilla ¹ Verde ² Bajo mersey ³	<i>Siderastrea siderea</i> ²	Dark band	Vcom, Vc, Vs	Vcom, Vc, Vs
			<i>Porites astreoides</i> ^{1,3}	Yellow spots	Vcom, Vs	
			<i>Montastraea cavernosa</i> ³	Dark band	Vcom	
			<i>Colpophyllia natans</i> ²	Bleaching	Ac	
Port Area, Veracruz	Vcom, Vc, Vs, Sm	Blanquilla ¹ Verde ² Bajo mersey ³	<i>Siderastrea siderea</i> ²	Dark band	Vcom, Vc, Vs	Vcom, Vc, Vs
			<i>Porites astreoides</i> ^{1,3}	Yellow spots	Vcom, Vs	
			<i>Montastraea cavernosa</i> ³	Dark band	Vcom	
			<i>Colpophyllia natans</i> ²	Bleaching	Ac	
Shipyards, Veracruz	Vcom	Gallega ¹ Galleguilla ² Blanquilla ³	<i>Siderastrea siderea</i> ²	Gray spots	-	Vcom
			<i>Porites astreoides</i> ^{1,3}	Yellow spots	Vcom, Vc	
Open Sky Channel El Cable	Vcom, Vc, Sm	Gallega ¹ Galleguilla ²	<i>Siderastrea siderea</i> ²	Gray spots	-	Vcom
			<i>Porites astreoides</i> ¹	Yellow spots	Vcom, Vc	

IV. Discussion

Pathogenic bacteria of the genus *Vibrio*, which were positive, occurred in 85% of PSP of analyzed samples, unlike coral samples where the percentage of bacteria decreased by 42.3%. According to the primer used HA-F/HA-R (*Vibrio complex*), this amplifies the gene encoding the zinc-metalloprotease, which is characteristic of pathogenic bacteria of the genus *Vibrio*, such as *V. cholera*, *V. vulnificus*, *V. harveyi*, *V. anguillarum*, *V. parahaemolyticus*, including the species of *V. coralliilyticus* and *V. shilonii* [16]. *Vibrio complex* in coral tissue suggests that some of the species mentioned above could be present. Therefore, it is possible that there is a risk of causing alterations to coral with diseases like White syndrome, Syndrome of Dark Spots, Black Band disease and Yellow Band disease [17][18][19][20].

Serratia marcescens bacteria had positive presence in six out of ten PSP sampling points. Its appearance in the marine environment may be due to the contribution coming from the PSP since this bacteria is common in the intestinal tract of humans and animals [21]. This same bacterium was not present in the tissue of the coral species *Colpophyllia natans*, *Montastraea cavernosa*, *Montastraea faveolata*, *Porites astreoides* and *Siderastrea siderea*. In studies, this bacterium is reported and known as pathogen of the coral genus *Acropora* and causes White Pox disease [22][23]. However, their

presence is not ruled out in other species, as the etiological characteristics of the disease in the coral *Acropora* are similar to other coral species.

The *Aurantimonas corallicida* bacteria only occurred in 11% of coral tissue *Colpophyllia natans*, *Siderastrea sidera* and *Montastraea cavernosa*, this bacterium is associated with a disease called White Plague; a sign of this disease is exposure of the limestone structure, showing no tissue in its periphery [24][25][26]. It was also found the presence of *A. Corallicida* and *Vibrio complex*, bacteria associated with the etiologic features of the aforementioned disease. So it is likely that this disease is caused by a bacterial complex and not just by the bacteria *Aurantimonas corallicida* in the previously mentioned coral species [27][28]. The genus *Oscillatoria* showed only in 4% of the tissue samples of analyzed coral, there were no etiologic lesions. Although there have been studies showing that it is a causative agent of the Black Band disease (BBD), which has affected more than 45 coral species.

The presence of pathogenic bacteria in different species of coral in the PNSAV, correspond to those identified in the PSP. By the distance of the reef with the PSP and according to the results obtained in this study, it reflects a potential risk for the contribution of pathogenic bacteria to the environment, which directly affect coral species. In 2005, it found a similar behavior due to the presence of diseases in corals that are located near to discharge zones [29]. Such is the case of the Veracruz Reef System National Park where these coral reefs found near urban coastal areas are exposed to excessive discharges of nutrients, sediment and pathogens that alter the survival of coral [30][31]. However, one of the affectations of greatest impact is the presence of pathogenic bacteria that cause diseases and threaten the ecological balance of the coral ecosystem [32].

V. Conclusion

The contribution of pathogenic bacteria from point sources of pollution is causing pathological affectations in corals, which are characteristic of the disease: White Band, Black Band, Yellow Band, White Plague and Bacterial Bleaching. This represents short-term negative impacts such as loss in coral cover, and long-term changes in the PNSAV faunal composition. However, the prevalence of pathogenic bacteria in corals could be related to the coral adaptation to these vulnerability conditions, this conclusion is reached since pathogenic bacteria were found in coral tissue without signs of injury or illness.

This problem represents potential risks to species of commercial interest that inhabit the reef, the above is a result of environmental impacts, loss of habitat for the species that live or depend on PNSAV, thus also reduction or migration of species, some modifications in life stage of the ecological niche and opportunities for invasive and opportunistic species. Socioeconomic impacts would be reflected in the decrease of the capture of species of commercial interest and an increment in prices as a result of fishing effort.

This study generated the first report of the presence of pathogenic bacteria from point sources of pollution and its direct effect on PNSAV coral reefs.

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