

Composition of the culturable bacterial community associated with the water column and soft tissues from oysters of the mangrove ecosystem at Honda Swamp, Colombian Caribbean

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Abstract

The study of bacterial communities is highly relevant in relation to the knowledge of global biological biodiversity and the biogeochemical cycles of the planet. Microbiological activity is the basis of ecosystems functioning, among these are the mangroves, which offer a wide variety of goods and services to humanity. Nevertheless, they have been severely impacted by anthropogenic activities such as felling, heavy metal pollution, water enrichment, overexploitation of resources, among others. This research aimed to characterize the culturable bacterial community from the water column and the oyster *Crassostrea rhizophorae* muscle tissue in a mangrove ecosystem from the Cartagena Bay, Colombian Caribbean as a baseline for future studies on bioremediation treatments. To accomplish this, four samplings were conducted over the course of a year, including contrasting climatic seasons (e.g., windy and rainy), collecting oyster individuals from mangroves and samples from superficial water. The bacterial community comprised 25 species, from which 9 were exclusive for the oyster tissue, 7 for the water column, and 9 generalists isolated from both types of samples. *Enterobacter cloacae* was the most frequent species during the year, followed by *Escherichia coli* and *Erwinia* sp. There were significant differences in the species composition between the sampling months (ANOSIM, $R = 0.492$; $p = 0.001$), because of the presence of a higher number of exclusive species in December than in the other months. Furthermore, the physicochemical variables that presented a major influence on the bacterial community composition were the wind velocity and the dissolved oxygen. The review of this research findings will allow to detect if any of the identified species could be exploited for bioremediation treatments, and it will contribute to the preservation of marine-coastal water bodies biodiversity.

Keywords: Bacterial community; bioremediation; *Crassostrea rhizophorae*; swamp; mangrove.

1. Introduction

The mangrove ecosystems offer many benefits such as support of productive and socio-cultural activities to maintain the biodiversity, in addition to the protection of the coastal populations from floods and winds caused by periodical storms (Beltrán and Suárez, 2016; Díaz *et al.*, 2010; Hawkins *et al.*, 2020). Mangroves provide energy and substrate to the ecosystem, and can also serve as habitat and shelter for a variety of organisms living in their trunks and between their roots.



Despite all their benefits, mangroves comprise one of the most threatened ecosystems, mainly by anthropogenic activities, especially the discharge of sewage with different types of chemical, organic, and inorganic wastes. Moreover, coastal development, population growth, agriculture, aquaculture, ecotourism, and other human activities also have a negative impact on the coast, thus threatening the associated flora and fauna and the health of the population (Beltrán and Suárez, 2016; Barbier, 2017; Umair, 2018).

Oysters are strongly associated with the entire mangrove system of the Honda swamp sector, and are considered one of the most appreciated edible shellfish (Graü *et al.*, 2004; Barbier, 2017) hence its economic importance to local communities, representing a relevant social context of the study area. Additionally, the oysters are filter-feeding organisms and bioaccumulate a large quantity of pathogenic bacteria, marine toxins, and traces of metals suspended in the water column (Lee and Morgan, 2003; Umair, 2018), responding quickly to environmental factors that occur in the water column, therefore, used as indicators of contamination and functional stress in coastal ecosystems (Lee and Morgan, 2003; Umair, 2018; Aguirre-Rubí *et al.*, 2018). Due to their biological and ecological characteristics, these oysters, when eaten by tourists and local, can become vectors of pathogens that generate significant impacts on public health, which has been widely evidenced (Potasman *et al.*, 2002; Elbashir *et al.*, 2018).

Microorganisms represent a very important group in the estuarine biota, since both bacteria and fungi take part in basic processes as organic matter decomposition and remineralization of nutrients (Alongi, 1988). Bacteria constitute 91 % of the microorganisms from mangrove swamps and have very important functions, including the removal of toxic H₂S, by transforming it into elemental sulfur; fixing N₂, which is one of the most relevant bacterial processes in mangroves, mainly in arid and semi-arid zones, decomposing rhizosphere, bark, and leaves; using the organic carbon dissolved from the sediments to prevent its export to adjacent systems like pelagic food chains or lagoon waters (Alongi and Boto, 1989), and participating in the absorption and retention of metals (Mendez *et al.*, 2005). In fact, microorganisms from the rhizosphere of *Laguncularia racemosa* and *Avicennia germinans* have been reported to generate organic acids that participate in the ion absorption in the root, possibly contributing to the tolerance of potentially toxic elements (Vásquez *et al.*, 2000), which has made them an important piece on bioremediation studies.

In addition, microbial diversity and their populations dynamics has become a research hot topic, especially in mangrove ecosystems, which has extended the knowledge about the composition of bacterial communities in contaminated soils, as well as their changes during biodegradation processes. However, there is not much information about the bacterial communities associated to the water column on mangrove estuaries and the edible organisms associated to these ecosystems. Therefore, the aim of this study is to characterize the culturable bacterial community from the water column and the oyster *Crassostrea rhizophorae* muscle tissue in a mangrove ecosystem from the Cartagena Bay, Colombian Caribbean as a baseline for future studies on bioremediation treatments.

2. Materials and methods

2.1. Study Area

The Honda swamp is located in the south-west sector of Cartagena Bay, across the industrial zone of the city (**Figure 1**), near the Puerto Bahía Port Society and the mouth of the Dique Channel. The water in the swamp is predominantly brackish due to the mixture of marine waters

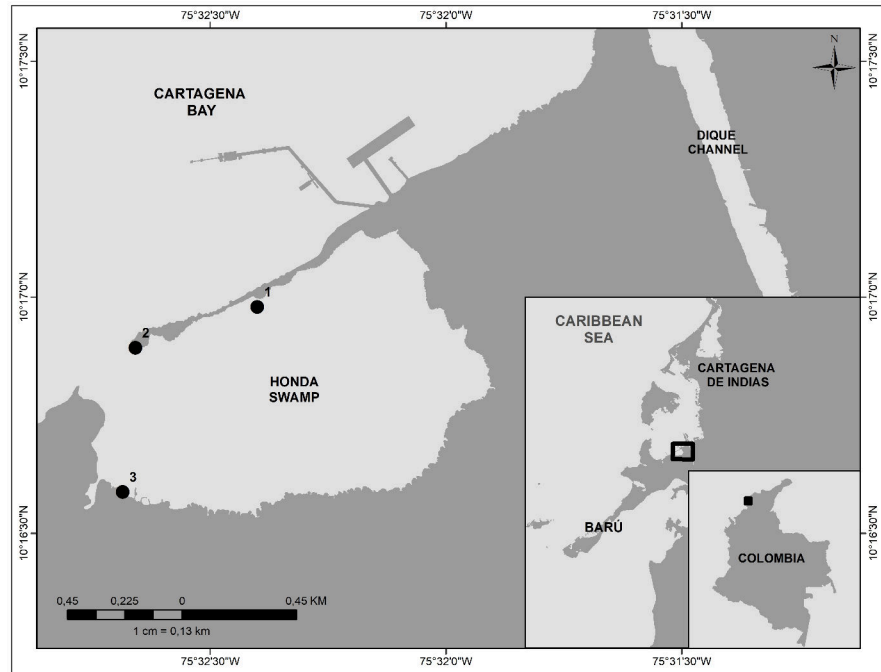


Figure 1. Map of the Honda Swamp. The black dots (1), (2), and (3), are the sampling stations.

that enter the bay with the fresh waters of the Dique Channel. The outer mangrove fringe is mainly composed by *Rhizophora mangle* (maximum height of 17 m) and, to a lesser extent, by *Laguncularia racemosa*, while *Avicennia germinans* and *Conocarpus erectus* can be found towards the internal mangrove (Alongi, 1988; Merchán, 2016).

The climatological features of Cartagena Bay are determined by trade winds in the dry season and by the north-south movement of the Intertropical Convergence Zone (ITCZ) in the rainy season, as occurs in the rest of the Colombian Caribbean (Franco-Herrera, 2005; Gutiérrez-Moreno *et al.*, 2012). Usually, the rainy season goes from August to November, with an increase in the rainfall, whereas the dry season extends from December to April, with the highest wind speeds of the whole year (Gutiérrez-Moreno *et al.*, 2012). In addition, there is a third climatic season known as the transition period between May and July, with weak winds, in which the “Veranillo de San Juan” develops, with an increase in ambient temperature, clear skies, increased sunlight and occasional rains (Franco-Herrera, 2005; Gutiérrez-Moreno *et al.*, 2012).

2.2. Sampling

Four samplings were carried out in three stations at the Honda swamp in four different months (March, May, September and December, 2018) (Figure 1). Two superficial water samples were taken per station in the morning hours (8:00 to 10:00 AM) following the protocols established in the NTC-ISO 5667-3:200 standard. In addition, 20 specimens of *Crassostrea rhizophorae* were collected using a sterile razor in the roots of the mangrove, achieving an average of 20 g of living tissue in the laboratory. Afterwards, the oyster shells were opened by cutting off the adductor muscles before the gills and muscle tissues were removed. The tissues of the 20 individuals were manually homogenized with a mortar and a pestle (Usese *et al.*, 2019). A total of 48 samples were obtained (24 from water and 24 from oysters), labeled, stored in refrigeration, and transferred to the laboratories of the Universidad del Sinú-Cartagena.

Measurements of salinity, temperature, pH, and dissolved oxygen in the water column were taken with a Thermo Scientific STARA 3260 multiparametric probe, and wind speed was measured using a GT8907 digital anemometer with a portable sensor, which had previously been calibrated according to the manufacturer's recommendations.

2.3. Isolation of the bacterial colonies

A culture dependent method was used to explore microbial diversity, as has been used to study the bacterial community composition associated with mangrove ecosystem (Gomes *et al.*, 2008; Peixoto *et al.*, 2011; Ghizelini *et al.*, 2012). Bacterial cultures were grown following the procedures outlined in Colombian Technical Standards NTC 947-1 and NTC 4458-2007, respectively.

After three serial dilutions of each sample, a culture in Plate Count® agar was made in order to isolate mesophilic aerobes, and incubated for 48 hours at $(35 \pm 2) ^\circ\text{C}$. A subculture was grown on nutritive Plate Count® agar to obtain a pure culture and incubated for 24 hours. After a Gram stain to validate the purity of the culture, the colony was inoculated in 0.5 mL of BHI broth with glycerol. The final cultures were stored frozen until identification (MINSALUD, 1981).

For total coliforms and *Escherichia coli*, a Chromocult® agar subculture was done and incubated for 24 hours. Once isolated, it was transferred to nutrient agar and incubated for another 24 hours. Subsequently, after a Gram stain to validate the purity of the culture, two to three colonies were selected and inoculated in 0.5 mL of BHI broth with glycerol. The final cultures were stored frozen until identification (MINSALUD, 1981).

2.4. Identification of the bacterial colonies

The bacterial strains were identified through biochemical tests such as Gram stain, Catalase-Oxidase, API 20E (for Enterobacteriaceae and others Gram-negative bacillus), API ENF® and BBL Cristal®. (Gram-positive bacteria). In addition, an automated identification was performed to confirm the identified bacterial strains, through a BD Phoenix 100™ which provides rapid, accurate and reliable detection of known and emerging antimicrobial species (BD Phoenix™ AP, 2022).

3. Data Analysis

3.1. Biological parameters

The obtained results were organized into a presence/absence species matrix using Excel 2019. A similarity ANOSIM test was carried out in order to verify if there were significant statistical differences between samples. Subsequently, a classification (dendrogram) and ordination (non-metric multidimensional scaling analysis-NMDS) analysis were performed, using the qualitative diversity index Jaccard, discriminating between sampling month, sites, and type of sample. Finally, in order to establish the species contribution to each factor (e.g., time, sites, and type of sample), a SIMPER test was applied. All the tests were executed using the software PRIMER-E v7 (Clarke and Gorley, 2015).

3.2. Abiotic parameters

In order to determine significant differences in the physicochemical parameters between sampling months, the Kruskal-Wallis test was applied (previous normality and homoscedasticity corroboration, with the Shapiro-Wilks and Levene tests, respectively) (Zar, 1996) and a Dunn Post-Hoc test for the parameters that presented significant differences, using the statistical software PAST 4.05 (Hammer *et al.*, 2001). Additionally, a Spearman correlation was carried out to see if there were any relationship between the abiotic parameters.

A principal component analysis (PCA) was carried out to determine the environmental parameters that provided a higher variability to the data, and to obtain a representation of the variable's ordination in relation to the sampling months. Finally, a biota environment matching test (BIOENV) was applied to establish the relationships between the physicochemical variables and the bacterial community, using the statistical software PRIMER-E v7 (Clarke and Gorley, 2015).

4. Results and discussion

4.1. Biological parameters

25 bacterial species were identified (**Table 1**), highlighting *Enterobacter cloacae*, found in 9 of the 24 samples analyzed (37.5 %), followed by *Escherichia coli*, which was registered in eight samples (33.3 %). From the water column, 18 species were isolated, 7 of them exclusive, and 17 species were isolated from oyster tissue, 9 of which were exclusive (Table 1). 10 of the reported species were identified on both types of samples.

Table 1. List of species isolated from water column and oyster tissue samples. The 'X' means presence.

Species	Water column	Oyster tissue
<i>Bacillus</i> sp		X
<i>Bibersteinia trehalosi</i>	X	X
<i>Chryseobacterium indologenes</i>	X	
<i>Chyseo bacterium versicularis</i>		X
<i>Citrobacter sedlakii</i>	X	
<i>Enterobacter cloacae</i>	X	X
<i>Enterococcus faecium</i>		X
<i>Erwinia</i> sp	X	X
<i>Escherichia coli</i>	X	X
<i>Klebsiella oxytoca</i>	X	X
<i>Klebsiella planticola</i>	X	
<i>Klebsiella</i> sp	X	X
<i>Ochrobactrum anthropi</i>		X
<i>Pantoea</i> sp		X

Table 1. List of species isolated from water column and oyster tissue samples. The 'X' means presence.

Species	Water column	Oyster tissue
<i>Proteobacterium danselae</i>	X	
<i>Proteus vulgaris</i>		X
<i>Pseudomonas luteola</i>	X	
<i>Pseudomonas oleovorans</i>	X	
<i>Rautella planticola</i>	X	X
<i>Rautella terrigena</i>	X	
<i>Serratia ficaria</i>	X	X
<i>Shewanella putrefaciens</i>	X	
<i>Staphylococcus epidermidis</i>		X
<i>Staphylococcus warnerii</i>	X	X
<i>Vibrio vulnificus</i>	X	X

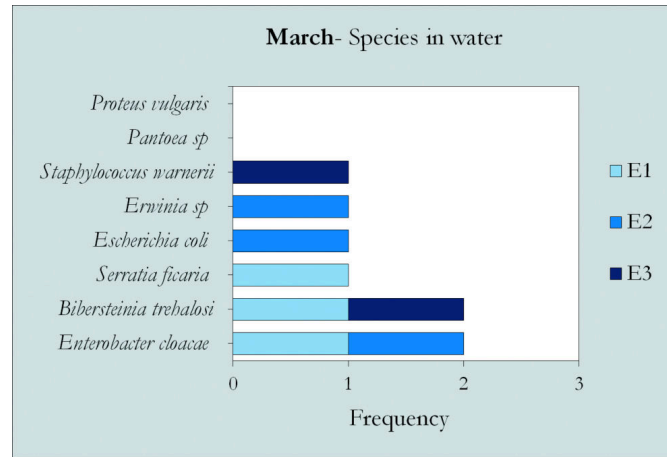
Seven species were identified in March, three of them were generalist (found in both water and oyster samples) (**Figure 2**). For the water samples, three exclusive species were identified. In addition, *E. cloacae* and *Bibersteinia trebalosi* had the highest frequency (two stations each) (**Figure 2(a)**). For the oyster tissue samples, *Erwinia* sp. and *E. cloacae* were the most frequent. Two exclusive species were also identified (**Figure 2(b)**).

Seven species were identified in May. *B. trebalosi* was isolated from all the stations. Five species were isolated from the water column, four of them exclusive. *Erwinia* sp. and *E. cloacae* were the most frequent (two stations) (**Figure 3**). As for the oyster tissue, two of the three species identified were exclusive.

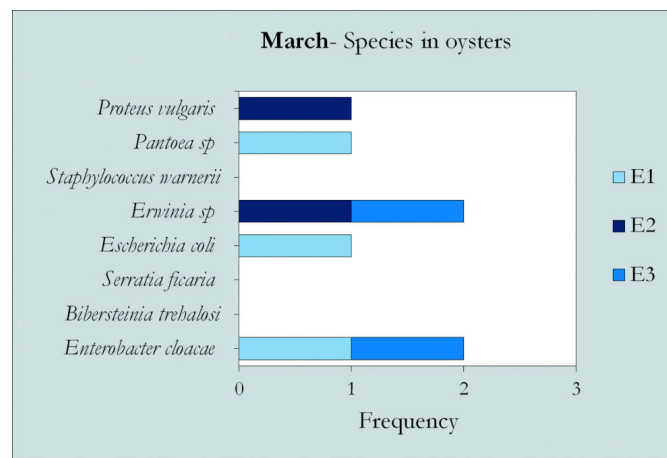
Eight species were identified in September, of which four were exclusive to the water column, three were exclusive to oyster samples, and one generalist species (*E. coli*). *E. cloacae* was isolated from the water column on each station, while the most frequent specie in oyster tissues was *Ochrobactrum antropi* (station 1 and 2) (**Figure 4**).

As for December, the highest species richness was observed ($S = 13$), seven of the identified species were exclusive to the water column, one was exclusive to the oyster samples, and five were generalist species (**Figure 5**). *E. coli* was present in the water samples of all the stations, while for the oyster samples, stands out *Rautella planticola*, found in stations 1 and 2 (**Figure 5**).

Some of the bacterial genus found in estuarine systems come from different anthropogenic activities that take place throughout the delta of the rivers conforming the estuary, mainly due to discharges of organic waste and untreated wastewater, affecting not only the water quality but also organisms with filtering characteristics such as bivalve mollusks (Bravo and Ortega, 2010). In addition, culture dependent count methods repeatedly indicate that microbial numbers decline in more heavily polluted sites and that communities change and functional groups enrich in response to an specific pollutant (Maciel-Souza *et al.*, 2006; Peixoto *et al.*, 2011).



(a)

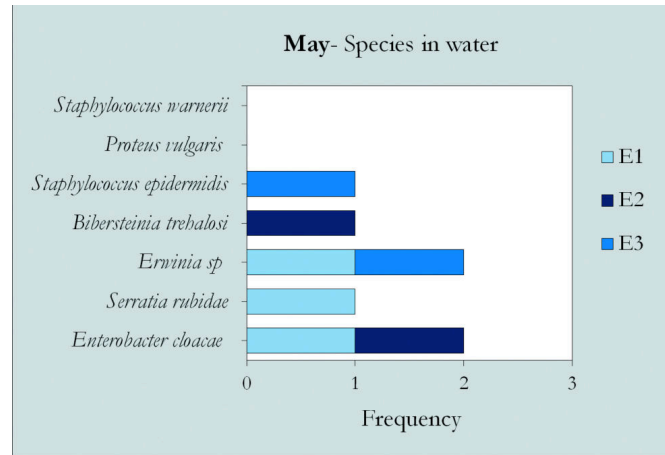


(b)

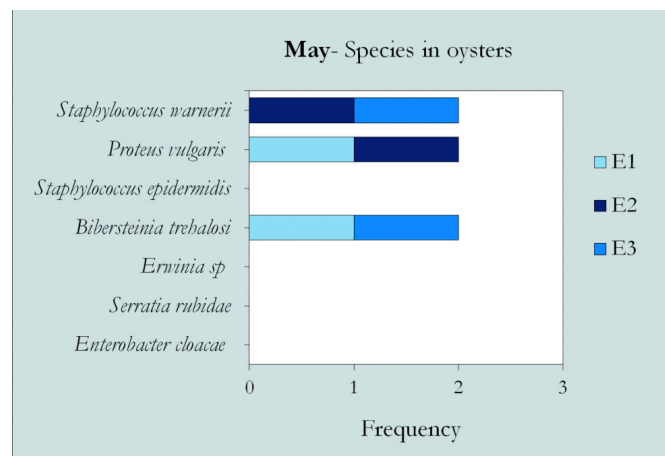
Figure 2. Species frequency (number of appearances) in March for (a) Water sample. (b) *Crassostrea rhizophorae* sample. The colors represent the stations.

Analyzing the species composition of the isolated bacteria, a similarity of less than 20 % was obtained between all the samples. The similarity cluster obtained by the Jaccard index, complemented with a SIMPROF analysis, showed the separation of the samples into two large groups, the first comprised by all the samples taken in December (water and oyster) and the oyster samples collected at station 3 in September, while the second group included the rest of the samples (May, March and most of September) (**Figure 6(a)**), this is corroborated by the non-metric multidimensional scaling analysis (NMDS) (**Figure 6(b)**).

The differences observed between the sampling seasons could be attributed to the contribution of the species (**Table 2**). For instance, in March, May, and September, *E. cloacae*, *Erwinia sp.*, and *Bibersteinia trehalosi* represented more than 50 % of the species identified. In contrast, in December, *E. coli*, *Klebsiella sp.*, and *Rautella planticola* contributed more than 70 % (**Table 2**). *E. cloacae* and *E. coli* contributed at least 50 % and 20 %, respectively, in every factor (month, site, type of sample) (**Table 2**). This could have been influenced by the strong seasonality of the study



(a)



(b)

Figure 3. Species frequency (number of appearances) in May for (a) Water sample. (b) *Crassostrea rhizophorae* sample. The color represents the stations.

area, given that March, May, and December, which are considered typically dry, had a higher species richness contrary to September, when the frequency of rainfall increases (Franco-Herrera, 2005; Gutiérrez-Moreno *et al.*, 2012).

Species from the Enterobacteriaceae family were frequently found during the study. One of these was *Enterobacter cloacae*, identified frequently in almost all months, except in December. This species is found in the gastrointestinal microbiota of humans and animals, and it is considered as an opportunistic pathogen that frequently colonizes hospitalized patients, primarily those receiving antibiotic treatment, and has been linked to secondary infection of burns and respiratory and urinary tract pathologies (Puerta and Mateos, 2010). However, it has been reported that *E. cloacae* has a high potential to remove heavy metals like mercury (Adelaja and Keenan, 2012), cadmium (Dash *et al.*, 2013) and chrome VI (Kavaruma and Esposito, 2010).

Raoultella planticola is a Gram-negative pathogen enterobacteria, usually reported in aquatic environments. This species can grow in high cadmium concentrations, precipitating insoluble cadmium sulfide, hence it can be used in cadmium bioremediation on exposed soils (Ershadi *et al.*, 2014). *E. coli*, also part of the Enterobacteriaceae family, reported in urinary (ECUP), respiratory

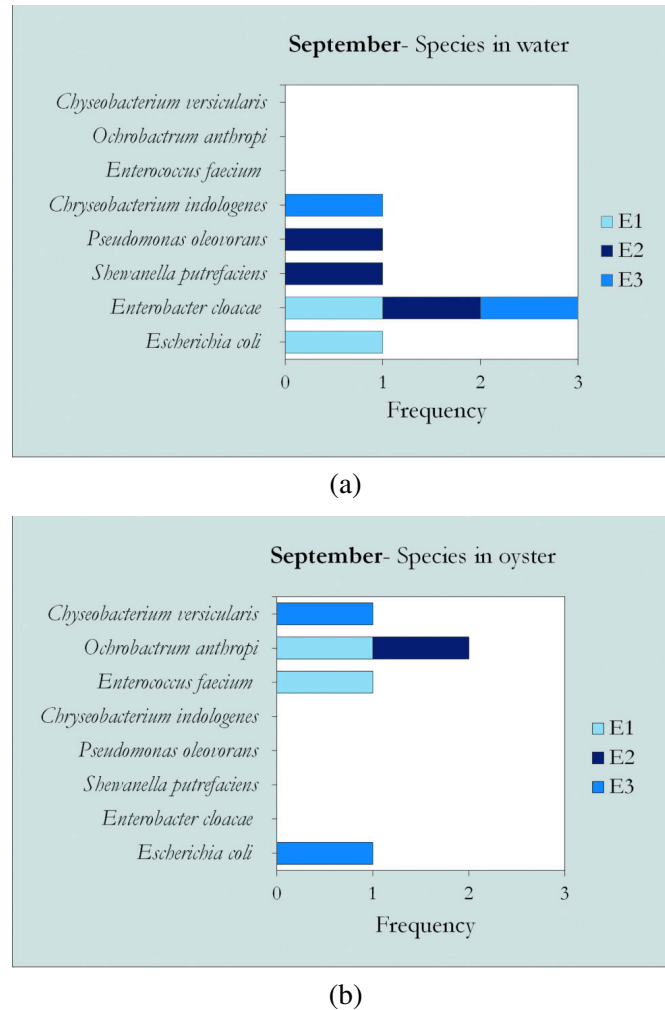
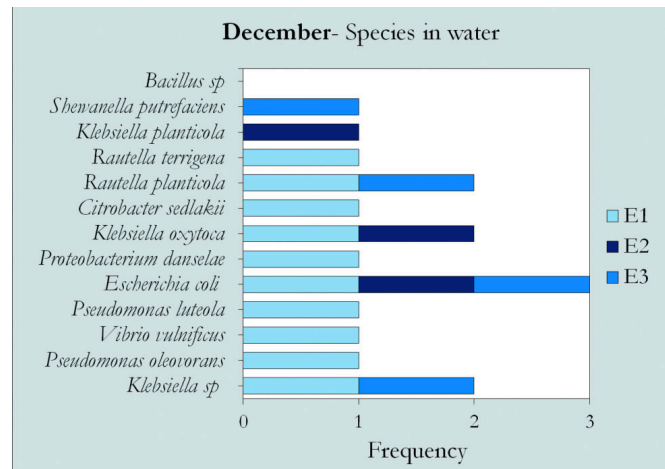


Figure 4. Species frequency (number of appearances) in September for (a) Water sample. (b) *Crassostrea rhizophorae* sample. The color represents the stations.

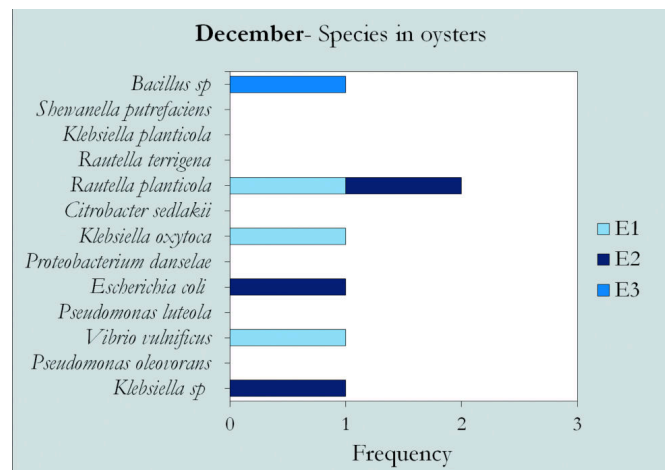
and central nervous system infections (Puerta and Mateos, 2010). Despite, this bacterial species can absorb heavy metals, tolerate different concentrations of chrome VI (up to 200 mg dm^{-m}) and can remove cadmium (Borremans *et al.*, 2002; Panigatti *et al.*, 2012; Khan *et al.*, 2015).

Table 2. SIMPER test- Contribution (%) of species discriminated by factors (month, site and type of sample). Species with a minimum of 70 % similarity between the groups.

Species	Sampling month				Sampling sites			Sample type	
	March	May	Sep	Dec	1	2	3	Water	Oyster
<i>Bibersteinia trehalosi</i>	-	51.72	-	-	-	-	19.48	-	22.5
<i>Enterobacter cloacae</i>	57.39	-	56.52	-	65.05	32.08	19.48	60.34	-
<i>Erwinia sp.</i>	33.91	17.24	-	-	-	-	25.97	-	-
<i>Escherichia coli</i>	-	-	21.74	32.44	21.3	28.3	-	20.56	17
<i>Klebsiella sp.</i>	-	-	-	14.23	-	-	-	-	-
<i>Proteus vulgaris</i>	-	17.24	-	-	-	14.15	-	-	22.5
<i>Rautella planticola</i>	-	-	-	35.69	-	-	-	-	17
<i>Staphylococcus warnerii</i>	-	-	-	-	-	-	19.48	-	-



(a)



(b)

Figure 5. Species frequency (number of appearances) in December for (a) Water sample. (b) *Crassostrea rhizophorae* sample. The color represents the stations.

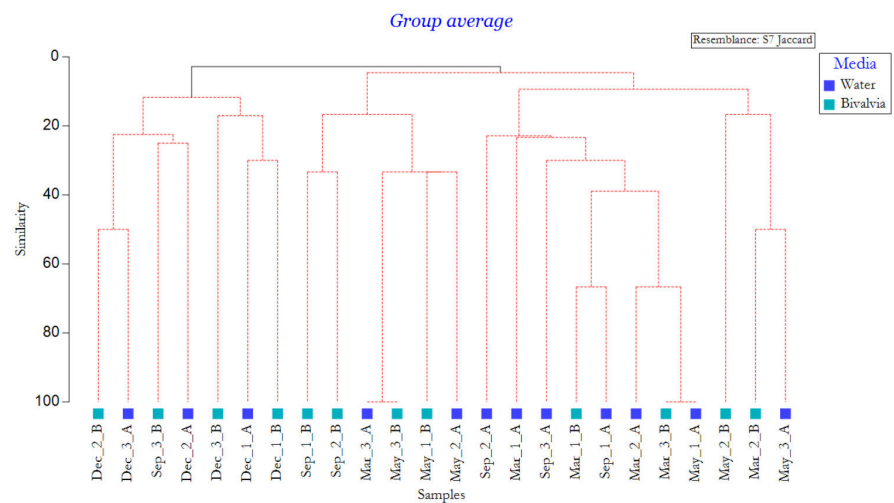
Proteus sp. was also frequently identified. Species from this genus are widely used in different types of industries, such as textiles, for their ability to remove colorants. A strain of *P. mirabilis*, isolated from sludge from a wastewater treatment plant achieved a percentage of discoloration between 13% and 17%, through biodegradation and bioadsorption mechanisms (Cortazar et al., 2012), while *Erwinia sp.* and *Vibrio sp.*, also frequent in the current research, are well-known as oil degraders (Trujillo and Ramírez, 2012), hence they could be used in bioremediation processes.

In a study carried out by González *et al.* (2009), different members of the Enterobacteriaceae family were isolated from water and oyster samples in three sampling points in Sucre State, Venezuela. Among the species identified are *Serratia rubidaea*, *Proteus spp.*, *Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter cloacae* and *Enterobacter intermedium*, similar to what was found in the present research, since the most frequently isolated genus in water samples were *E. cloacae* and *E. coli*, and in oyster samples, *E. coli* and *Proteus vulgaris*.

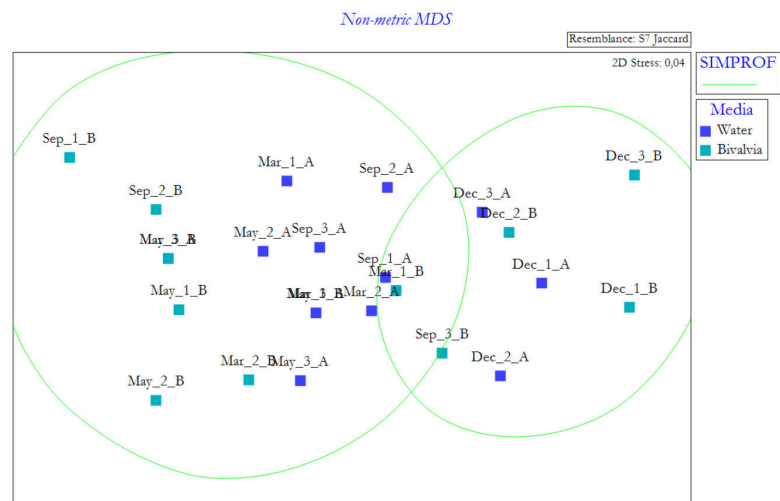
4.2. Abiotic parameters

The water body was characterized by an average temperature of $(30.85 \pm 0.48) ^\circ\text{C}$, a salinity value between 19 ppt and 28.41 ppt, the pH evidenced a slightly basic environment (8.12 ± 0.06) , and the dissolved oxygen (DO) ranged between 1.8 mg dm^{-3} and 7.63 mg dm^{-3} . Furthermore, during the sampling year, the average wind speed was $(14.0 \pm 20.6) \text{ km h}^{-1}$. The lowest values were observed in May for the salinity parameters (19 ppm), while in March the most critical value of dissolved oxygen was 1.8 mg dm^{-3} . The highest salinity value was obtained in September (28.41 ppt) while the highest DO was obtained in December (7.63 mg dm^{-3}) (**Table 3**).

The Kruskal-Wallis analysis showed significant differences between the sampling months in ambient temperature, salinity, wind speed, and dissolved oxygen ($p < 0.05$) (**Table 4**). Ambient temperature showed differences between March-May and March-September (Dunn's Post-Hoc



(a)



(b)

Figure 6. Classification and ordering analysis of the bacterial community composition from the Jaccard index. (a) Cluster (b) NMDS. Cophenetic index 0.794. Both graphics show significant differences between the group of samples taken in December from the samples taken in March, May and September.

Table 3. Environmental conditions recorded during the four samples taken at the three Honda Swamp stations. Temperature of water (T water), Ambient Temperature (AT), Salinity (Sal), Wind Speed (WS), Dissolved Oxygen (DO), and pH. SE=Standard Error.

Month	T water (°C)	SE	AT (°C)	SE	Sal (ppt)	EE	WS (km/h)	EE	DO (mg/dm)	SE	pH	SE
Mar	31.00	2.00	29.10	0.00	20.67	0.33	19.30	0.00	1.80	0.00	8.00	0.00
May	30.50	0.79	32.13	1.62	19.33	0.33	4.80	0.00	7.33	0.33	8.23	0.23
Sep	31.57	0.27	32.50	0.49	28.41	3.01	14.23	4.89	6.91	0.53	8.20	0.02
Dec	30.37	0.19	29.53	0.18	21.68	0.61	19.30	0.00	7.63	0.00	8.05	0.10

$p < 0.05$). Regarding salinity, the main differences were observed in May and September (Dunn's Post-Hoc $p < 0.05$), the lowest values were recorded in May, (19.33 ± 0.33) ppt, while the highest values were obtained in September, (28.41 ± 3.01) ppt (Table 3). In addition, the wind speed showed the main differences between March-May and May-December (Dunn's Post-Hoc $p < 0.05$). Dissolved oxygen presented significant differences between the months of March and December, with the lowest value of the year in the first month, and the highest in the last (Dunn's Post-Hoc $p < 0.05$) (Table 3).

The Spearman correlation showed a highly significant negative correlation ($R = -0.74$; $p = 0.0056$) between wind speed and salinity. Likewise, non-significant correlations were found between wind speed and dissolved oxygen ($R = 0.29$; $p = 0.3534$) and wind speed and pH ($R = -0.38$; $p = 0.2213$). These relationships could be explained by the water mix produced by a high wind speed (Niu *et al.*, 1998), which allows a greater interchange between the Honda swamp and the Cartagena Bay.

The principal component analysis (PCA) showed that components 1 and 2 had an influence of 88.2% on the abiotic data variation, highlighting the wind speed, salinity, and dissolved oxygen (Table 5). The months of March and December are characterized by greater variations in wind speed, while September is more influenced by salinity and dissolved oxygen (Figure 7). This could also explain the separation obtained in Jaccard's similarity cluster, in which the separation of the community is observed, between the months considered typically dry (March-May-December) and the month of September, considered rainy.

The BIO-ENV analysis results showed a high influence of wind speed ($r = 0.896$), which could be associated with sediment resuspension processes, as well as internal swell, light tides, and vertical mixing processes and stratification of the water column (Pinilla *et al.*, 2010; Gil-Aguilar *et al.*, 2013), generating an increase in nutrients and influencing the composition of the bacterial community, mainly in the typically dry months (March, May, and December) where the wind

Table 4. Kruskal-Wallis analysis by parameter between months. Temperature of water (T water), Ambient Temperature (AT), Salinity (Sal), Wind Speed (WS), Dissolved Oxygen (DO), and pH.

Kruskal-Wallis	T water (°C)	AT (°C)	Sal (ppt)	WS (km h ⁻¹)	DO (mg dm ⁻³)	pH
H (χ^2)	2.59	8.231	9.397	6.577	7.821	4.59
Hc (tie corrected)	2.599	8.348	9.497	7.615	8.075	4.953
p (same)	0.4577	0.03935	0.02336	0.05467	0.04449	0.1752

Table 5. Eigenvalues, percent of the total variance (% Variance), and percent of cumulative variation (Cum. % Variation) explained of the first five principal components (PC) of the principal component analysis (PCA) on environmental variables.

PC	Eigenvalues	% Variation	Cum. % Variation
1	54.4	65.3	65.3
2	0.2	22.8	88.2
3	4.7	5.7	93.8
4	2.9	3.5	97.3
5	2.2	2.6	100

speed is higher due to the presence of trade winds (Franco-Herrera, 2005; Gutiérrez-Moreno *et al.*, 2012). This variable, combined with dissolved oxygen and salinity, reaches $r = 0.992$, which is why these are considered the parameters that best explain the data obtained from the bacterial community, thus complementing what was observed in Kruskal-Wallis and PCA.

The physicochemical characteristics reported in this research (Table 3) are consistent with previous reports. According to Pinilla *et al.* (2010), the Honda swamp is part of the Bajo Canal del Dique swamp complex, which presents an acceptable limnological state (52.95). This is supported by dissolved oxygen values of 6 mg dm^{-3} and $\text{pH} = 7.99$ and, in addition, it has been highlighted as an area of fishing importance for local people. This acceptable limnological state makes Honda Swamp an important site for the region and, therefore, research must continue to monitor the physicochemical and biotic conditions (Hemraj *et al.*, 2017).

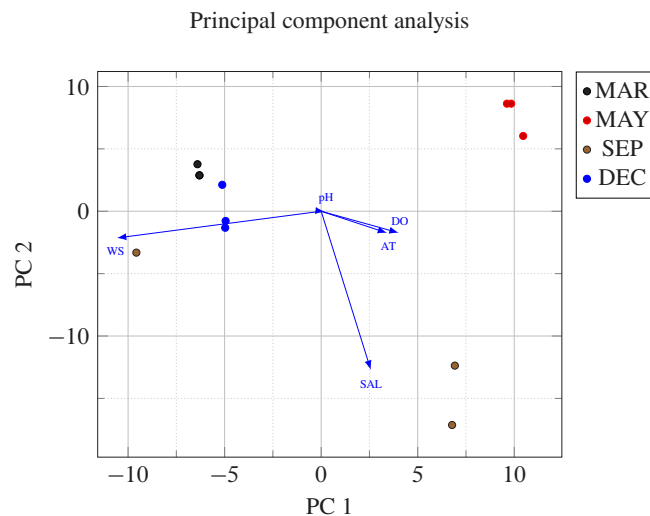


Figure 7. Principal Component Analysis (PCA). Water temperature (Water Temp), Ambient Temperature (Amb Temp), Salinity, Wind Speed, Dissolved Oxygen. The graphic shows that wind speed had a higher contribution with the results obtained in March, May and Dec, while September was more influenced by the salinity.

5. Conclusion

This research shows the firsts insight of the culturable bacterial community associated to a mangrove ecosystem in the Colombian Caribbean. The most frequent species were *Enterobacter cloacae*, followed by *Escherichia coli* and *Erwinia* sp. Moreover, the climatic pattern of the study area was proven to be a change driver for the community.

In addition, the detected variations in the species composition suggested a strong anthropogenic influence in the mangrove ecosystem, related to the high frequency of human pathogens, such as *Enterobacter cloacae*, *E. coli*, *Klebsiella* sp. and *Raoultella planticola*. However, these same species, including *Proteus* spp. and *Erwinia* sp. shows the potential of Honda swamp as a study field for bioprospection and bioremediation, since these species had been reported as oil removers, have a high potential to remove heavy metals like mercury, cadmium and chrome VI, and also, have been used in wastewater treatment plants.

Finally, the review of this research findings, as well as its continuation in further studies, will allow to exploit some of the identified species to develop biological bioremediation methods to reduce or eliminate sources of contamination, and it will help contribute to the preservation of marine-coastal water bodies biodiversity.

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Composición de la comunidad de bacterias cultivables asociada a la columna de agua y tejidos blandos de ostras del ecosistema de manglar en el pantano Honda, Caribe Colombiano

Resumen: El estudio de las comunidades bacterianas es altamente relevante y se relaciona con el conocimiento de la biodiversidad biológica global y los ciclos biogeoquímicos del planeta. La actividad microbiológica es la base del funcionamiento de los ecosistemas, entre los que se encuentran los manglares, que ofrecen una amplia variedad de bienes y servicios a la humanidad. Sin embargo, éstos se han visto altamente afectados por actividades antropogénicas como la tala, la contaminación por metales pesados, el enriquecimiento del agua, la sobreexplotación de recursos, entre otras. Esta investigación tuvo como objetivo caracterizar la comunidad bacteriana cultivable de la columna de agua y el tejido muscular de la ostra *Crassostrea rhizophorae* en un ecosistema de manglar de la Bahía de Cartagena, Caribe colombiano como línea de base para futuros estudios sobre tratamientos de biorremediación. Para lograr esto, se realizaron cuatro muestreos en el transcurso de un año, incluyendo estaciones climáticas contrastantes (por ejemplo, viento y lluvia), y se recolectaron individuos de ostras de manglares y muestras de agua superficial. La comunidad bacteriana comprendió 25 especies, de las cuales 9 eran exclusivas para el tejido de la ostra, 7 para la columna de agua y 9 generalistas, aisladas de ambos tipos de muestras. *Enterobacter cloacae* fue la especie más frecuente durante el año, seguida de *Escherichia coli* y *Erwinia* sp. Hubo diferencias significativas en la composición de especies entre los meses de muestreo (ANOSIM, $R = 0.492$; $p = 0.001$), debido a la presencia de un mayor número de especies exclusivas en diciembre que en los otros meses. Adicionalmente, las variables fisicoquímicas que tuvieron mayor influencia en la composición de la comunidad bacteriana fueron la velocidad del viento y el oxígeno disuelto. La revisión de los resultados de esta investigación permitirá detectar si alguna de las especies identificadas podría ser explotada para tratamientos de biorremediación, y contribuir a la preservación de la biodiversidad de cuerpos de agua marino-costeros.

Palabras Clave: Comunidad bacteriana; Biorremediación; *Crassostrea rhizophorae*; Pantano; Manglar.

Composição da comunidade de bactérias cultiváveis associadas à coluna de água e tecidos moles de ostras do ecossistema de manguezais no pântano de Honda, Caribe colombiano

Resumo: O estudo das comunidades bacterianas é altamente relevante e relaciona-se com o conhecimento da biodiversidade biológica global e dos ciclos biogeoquímicos do planeta. A atividade microbiológica é a base para o funcionamento dos ecossistemas, incluindo os manguezais, que oferecem uma ampla variedade de bens e serviços à humanidade. No entanto, estes têm sido altamente afetados por atividades antropogênicas, como exploração madeireira, poluição por metais pesados, enriquecimento da água, superexploração de recursos, entre outros. Este trabalho teve como objetivo caracterizar a comunidade bacteriana cultivável da coluna de água e do tecido muscular da ostra *Crassostrea rhizophorae* em um ecossistema de manguezais da Baía de Cartagena, Caribe colombiano, como linha de base para futuros estudos sobre tratamentos de biorremediação. Para conseguir isso, foram realizadas quatro amostragens ao longo de um ano, incluindo estações meteorológicas contrastantes (por exemplo, vento e chuva). Adicionalmente, indivíduos de ostras de mangue e amostras de água superficial foram coletados. A comunidade bacteriana esteve composta por 25 espécies, das quais 9 eram exclusivas para o tecido de ostra, 7 para a coluna d'água e 9 generalistas, isoladas dos dois tipos de amostras. *Enterobacter cloacae* foi a espécie mais frequente durante o ano, seguida por *Escherichia coli* e *Erwinia* sp. Houve diferenças significativas na composição de espécies entre os meses de amostragem (ANOSIM, $R = 0.492$; $p = 0.001$), devido à presença de um maior número de espécies exclusivas em dezembro do que nos outros meses. Além disso, as variáveis físico-químicas que tiveram maior influência na composição da comunidade bacteriana foram a velocidade do vento e o oxigênio dissolvido. A revisão dos resultados desta pesquisa permitirá discernir se alguma das espécies identificadas poderia ser explorada para tratamentos de biorremediação e contribuir a preservação da biodiversidade dos corpos d'água marinho-costeiros.

Palavras-chave: Comunidade bacteriana; Biorremediação; *Crassostrea rhizophorae*; Pântano; Manguezal.

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