DIVERSITY OF BACTERIA ASSOCIATED WITH THE MANGROVE FIDDLER CRAB, *Uca rapax*, IN BOQUERÓN, PUERTO RICO AND THEIR CELLULOSE DEGRADATION CAPACITY

by

ANGÉLICA M. OLMO-FONTÁNEZ

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Biology

UNIVERSITY OF PUERTO RICO

MAYAGÜEZ CAMPUS

2013

Approved by:

_____	_____
Rafael Montalvo Rodríguez, PhD Member, Graduate Committee	Date
_____	_____
Carlos J. Santos Flores, PhD Member, Graduate Committee	Date
_____	_____
Matías J. Cafaro, PhD President, Graduate Committee	Date
_____	_____
Nanette Diffoot Carlo, PhD Chairperson of the Department	Date
_____	_____
Áurea Rodríguez, PhD Representative of Graduate Studies	Date

DIVERSITY OF BACTERIA ASSOCIATED WITH THE MANGROVE FIDDLER CRAB, *Uca rapax*, IN BOQUERÓN, PUERTO RICO AND THEIR CELLULOSE DEGRADATION CAPACITY

ABSTRACT

Mangrove habitats are considered one of the most productive ecosystems in the world, and are very important because they are habitats for many microorganisms and marine invertebrates, such as fiddler crabs. Fiddler crabs (*Uca rapax*) are small deposit-feeders crustaceans, hence the particles found in mangrove sand and especially detritus are part of their diet. Their primary ecological role is the recycling of organic matter; also by burrowing deep into the mud, they create tunnels that aerate the estuarine soil, what promotes aerobic conditions. Mangrove soils contain mainly lignocellulose components, cellulases are secreted by soil microorganisms, yet it is not known if fiddler crabs's gut microflora is able to do so also. The gut microbes might contribute to the host nutritional fitness, especially by increasing the extracellular enzymes to degrade organic matter, such as lignocellulose. First, we compared the bacterial communities from mangrove soil and the hindgut microflora of fiddler crabs during the wet and dry seasons using culture-dependent methods (Chitin media, Lenox broth (LB) media, Marine agar and Mangrove soil agar). Bacterial isolates were then identified through 16S rDNA gene amplification and sequencing. UniFrac analysis found significant differences between mangrove soil and hindgut microflora bacterial communities only in the wet season. To identify specific bacterial communities associated with the hindgut of *Uca rapax*, we compared the genera present there with those in the soil. *Vibrio* was the only genus that was consistently isolated from the hindgut (in both seasons), but never detected in mangrove soil; although it was isolated at a low frequency (3.8%). We studied cellulose degradation, using carboxymethylcellulose (CMC) as carbon source in order to identify cellulase producing bacteria associated with the hindgut of *Uca rapax*; where more than 80% were positive cellulase producers under laboratory conditions. The statistical analysis showed significant differences in cellulose degradation capacity among bacterial strains suggesting that some strains have different enzymatic capabilities when degrading cellulose. Our results could suggest that the hindgut microflora of *Uca rapax*, is involved in the recycling of carbon. This implies that the activity of cellulases makes an important contribution to the nutrition by converting cellulose into simpler carbohydrates prior to the ingestion of detritus by the fiddler crabs.

DIVERSIDAD DE LAS BACTERIAS ASOCIADAS AL CANGREJO VIOLINISTA DE MANGLE, *Uca rapax*, EN BOQUERÓN, PUERTO RICO Y SU CAPACIDAD PARA DEGRADAR CELULOSA

RESUMEN

Los manglares son considerados uno de los ecosistemas más productivos del mundo. Poseen gran importancia ecológica debido a que sirven de hábitats para microorganismos e invertebrados marinos, como los cangrejos violinistas. Los cangrejos violinistas (específicamente la especie *Uca rapax*) son pequeños crustáceos detritívoros, lo cual implica que se alimentan del sedimento del manglar, específicamente del detrito. Su rol ecológico es reciclar materia orgánica cuando filtran y crean túneles en el suelo, que a su vez permiten aireación del mismo. El suelo del mangle está compuesto principalmente de derivados lignocelulíticos, pero se desconoce si la producción de celulasas necesaria para obtener los nutrientes del suelo son secretadas por los cangrejos así como por las bacterias. No obstante, las bacterias asociadas al sistema intestinal deben contribuir al desempeño nutricional del cangrejo, particularmente por la producción de enzimas extracelulares que degraden materia orgánica (lignocelulosa). Inicialmente, comparamos las comunidades bacterianas del sedimento y del intestino posterior de *Uca rapax* tanto en época húmeda como en época seca utilizando métodos dependientes de cultivo (medios de quitina, Lenox (LB), agar marino y sedimento de mangle) y luego amplificamos y secuenciamos el gen del 16S rADN. Los análisis realizados en UniFrac presentaron diferencias significativas entre las comunidades bacterianas aisladas del sedimento y del intestino posterior únicamente en la temporada húmeda. Para identificar comunidades específicas de bacterias asociadas al intestino posterior de *Uca rapax*, comparamos los géneros presentes en ambos ambientes. *Vibrio* fue el único género siempre aislado del intestino posterior en ambas temporadas, pero nunca se aisló del suelo del manglar. A pesar de que *Vibrio* se aisló consistentemente, su frecuencia fue baja (3.8%). Realizamos un estudio de degradación de celulosa, utilizando carboximetilcelulosa (CMC) como fuente de carbono, y los resultados indican que en ambas temporadas, más del 80% de las bacterias analizadas del intestino posterior fueron productoras de celulasas bajo condiciones de laboratorio. Los análisis estadísticos establecen diferencias significativas en la producción de celulasas, lo cual sugiere que algunas cepas poseen diferentes capacidades para degradar celulosa. Nuestros resultados sugieren que las comunidades de bacterias asociadas al intestino posterior de *Uca rapax* pueden estar involucradas en el reciclaje de carbono. Esto implica que la actividad enzimática de celulasas compone una importante contribución en la nutrición, cuando convierten celulosa a carbohidratos simples antes de la ingestión del detrito por los cangrejos violinistas.

DEDICATION

With all my gratitude…

I dedicate this work to God for bringing me light when the way seemed dark.

To all of those who contributed in my development as a microbiologist.

Especially to my parents, Carmen M. Fontánez and Antonio Olmo

for their unconditional love and guidance.

And to my husband, Carlos Solano, for being my support and my heart

despite the distance…

ACKNOWLEGDEMENTS

Especially, I want to express my gratefulness to my advisor and mentor, Dr. Matías J. Cafaro, for his unconditional commitment, patience, motivation and guidance. Also, I want to thank the members of my committee: Dr. Rafael Montalvo and Dr. Carlos Santos for their help and support. I would like to thank Mr. José Almodóvar for his help with some microscopy pictures and Mrs. Magaly Zapata for her guidance about on microbiological techniques.

I want to thank the group of graduate students from the Biology Department at UPRM that helped and supported me since I began the master's degree, especially Mariely Medina and Ginamary Negrón. And also all the members of the Symbiosis Laboratory for their constant help and good experiences; especially the undergraduate students: Verónica Figueroa, Mayrim Millán, Keyla Rivera and Lourdes Irrizarry, for their collaboration in the lab and in the field. I would like to acknowledge all the people from the Biology Department that supported me during this process and the National Science Foundation (NSF) Award MCB-0702025 for financial support.

Last but not least, I would like to thank all my family for their unconditional support, love and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Chapter 1: General Overview

1.1 Introduction

Mangrove habitats are considered one of the most productive and irreplaceable ecosystems in the world. According to Kathiresan et al. (2001), they are defined as woody plants that grow at the interface between land and sea in tropical and subtropical latitudes. Mangroves permit the nourishment, protection and reproduction for many species. Their conservation is very important because they are habitats for many organisms, such as bacteria, fungi, archaea, algae, plants, insects, and marine invertebrates, among others. In addition, some of these organisms help increase productivity and conservation of these ecosystems. For example, bacteria can regulate the chemical environment of sediments by transforming essential nutrients, such as carbon, nitrogen and phosphorus. They are the responsible of the degradation and recycling of essential elements of all of Earth's ecosystems, especially through extracellular enzyme production, such as lignocellulases (McCarthy, 1987). Bacteria also play an important role as mineralizers of organic detritus in mangrove ecosystems, which is essential food for protists and marine invertebrates (Alongi, 1994). According to these ideas, bacteria may play several roles that permit the stability of mangrove ecosystems and of the organisms that live in them.

Despite that mangrove forests exist under conditions of high salinity, high temperature and anaerobic soils, mangrove fauna can contribute in adding oxygen to sediments. The primary responsible invertebrates are fiddler crabs, which contribute to the maintenance of mangroves by building their burrows and enabling water to penetrate the substratum; hence, they provide an oxygen-rich environment for mangrove roots (Zeil et al. 2006). Fiddler crabs or mudflat crabs

are semi-terrestrial marine invertebrates, which have a widespread distribution and are active in mangroves and coastal marshes ecosystems (Crane, 1975). They are surface deposit feeders; typically consume detritus, wood or other plant materials, which implies the use of lignocellulose as carbon source. Cellulase activity has been reported in many marine invertebrates, but it is not clear whether degradation of cellulose can be carried out by the host, by their associated microorganisms or by both (Dall et al. 1983). This strongly suggests that the fiddler crabs should have symbiotic microflora associated to their guts to assist in degrading complex polymers (Harris, 1993), especially the lignocellulosic rich fraction of their diet. Therefore, it could be assumed that there is a direct relationship between bacterial abundance and enzyme activity associated with fiddler crab hindgut exists. According to Lau et al. (2002), bacteria in the hindgut of marine invertebrates are not competing directly with their host for uptake of digested compounds, which means that in the midgut segment is where the main absorption of nutrients by the host occurs.

Moreover, this relationship between bacteria and fiddler crabs might represent a mutualistic nutritional symbiosis. Little is known about nutritional symbiosis in marine invertebrates, but this nutritional association can suggest several benefits for the host's fitness and also for the symbionts. In a few words, the cooperative participation between bacteria and fiddler crabs can provide adaptive and survival advantages and it can also lead to many benefits for the conservation and stability of mangroves.

1.2 Literature Review

1.2.1 Mangroves, an irreplaceable ecosystem

Mangrove ecosystems dominate at the interface between land and sea, covering the 60-70% of world's tropical and subtropical coastlines (Thatoi et al. 2013). They are known as salty forests because they exist in conditions of high salinity, extreme tides, strong winds, high temperatures and muddy, anaerobic soils; therefore, their components are well-adapted to their natural conditions (Kathiresan et. al. 2001). Mangrove ecological roles include erosion and flood control, retention of toxic substances avoiding water contamination, source of organic matter, biomass export and storm protection, among others. Mangrove forests are considered one of the most productive and irreplaceable ecosystems of the world with biological processes having a major role in soil development and control on accretion and elevation change (Nyman et al., 2006). The cycles of matter are driven by physical (daily tides and rainfall) and biological (leaf fall and microbial decomposition) processes that control the rate of import and export of inorganic and organic compounds (Lugo et al. 1974). Stress factors could affect the essential structural and functional composition of mangrove ecosystem. The potential stress factors result from a mixture of man's activities and natural factors such as channelization, drainage, siltation, herbicides and hurricanes (Lugo et al. 1974). Unfortunately, the human encroachment due to diversion of freshwater for irrigation, land reclamation, and aquaculture has destroyed extensive mangrove forests around the world (Kathiresan et. al. 2001). One of the most important reasons to conserve mangrove ecosystem is because several species are at high risk of extinction, what has important ecological and economic consequences. For example, water purification services provided by mangrove species in the Muthurajawela Marsh, Sri Lanka, are valued at more than $US 1.8 million per year (Polidoro et al. 2010).

In the south-western region of Puerto Rico, there are only four mangrove species present: *Rhizophora mangle, Laguncularia racemosa, Avicennia germinans* and *Conocarpus erectus*, the first two mentioned being the most abundant (DRNA 2008). In the sampled area, the mangrove species *Avicennia germinans* (Spanish common name is "*Mangle Negro*") dominates. For the species *Rhizophora mangle* (Spanish common name is "*Mangle Rojo*") and *Laguncularia racemosa* (Spanish common name is "*Mangle Blanco*"), a global loss of 17% has been reported, but their red list category is *Least Concern* (Polidoro et al. 2010).

1.2.2 Mangrove-associated bacteria

According to Kathiresan et al. (2001), mangroves provide a unique ecological environment (due to the abundance of carbon and other nutrients contents) for diverse bacterial communities; and they are fundamental for the proper functioning of these habitats. Mangrove bacteria are one of the principal components in the mangrove sediments that maintain the productivity of these ecosystems. Microbes in tropical ecosystems, in particular, are even more productive and efficient in recycling nutrients than their counterparts in higher latitudes, indicating that they are proportionately more important in terms of energy flow in low latitude ecosystems (Alongi, 1994). The bacterial mangrove community regulates its chemical environment, particularly, transforming nutrients such as carbon, nitrogen and phosphate through photosynthesis, methanogenesis, nitrogen fixation, improve phosphate solubility, sulfate reduction and the production of other substances including antibiotics and enzymes (Santos et. al. 2011). The frequent bacterial groups which are involved in regulating the mangrove ecosystem are: sulfate-reducing (*Desulfovibrio*, *Desulfotomaculum*, *Desulfosarcina*,

Desulfococcus, and others), N2-fixing (*Azospirillum*, *Azotobacter*, *Rhizobium*, *Clostridium*, *Klebsiella* and others), phosphate-solubilizing (*Bacillus*, *Paenibacillus, Xanthobacter, Vibrio proteolyticus*, *Enterobacter*, *Kluyvera*, *Chryseomonas* and *Pseudomonas*), photosynthetic anoxygenic (*Chloronema*, *Chromatium*, *Beggiatoa*, *Thiopedia*, *Leucothiobacteria*, etc.), methanogenic (*Methanoccoides methylutens*, etc.), secondary metabolites-producing (Actinobacteria such as: *Actinomadura*, Microbispora, *Nonomuraea*, *Actinoplanes, Micromonospora*, *Verrucosispora*, *Arthrobacter*, *Isoptericola*, *Micrococcus*, *Microbacterium*, *Nocardia*, *Rhodococcus* and *Streptomyces*) and cellulose-degrading (*Bacillus* and *Cellulomonas*) bacteria (Thatoi et al. 2013). The decomposition and reconstitution of this organic matter leads to reestablishment of the ecosystem and, even more, of the biosphere.

Utilizing a cultivation-independent molecular approach to investigate the bacterial diversity in the surface sediments of a subtropical mangrove ecosystem in Shenzhen, China, Liang et al. (2007) found that the bacterial community is dominated by Proteobacteria (gamma, epsilon and delta), Cytophaga–Flexibacter–Bacteroides group, Actinobacteria, Chloroflexi and Firmicutes, among others. Other investigations about mangrove sediment in Sundarban-India revealed similar bacterial phyla: Proteobacteria (alpha, beta, gamma, and delta), Flexibacteria (CFB group), Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, Planctomycetes and Gammatimonadates (Ghosh et al. 2010).

Is important to mention that some mangrove bacteria can live symbiotically with other organisms, such as marine invertebrates. They can be transients or residents (symbionts) in the digestive system of the host, the presence of which have different implications for the invertebrates (Harris, 1993).

1.2.3 Mangrove-associated fiddler crabs

In mangroves, we also find other organisms, such as fiddler crabs that in addition to bacteria, are equally important ecological species. Crabs contribute to the maintenance of mangroves by building burrows that allow water to penetrate the substratum providing an oxygen-rich environment for mangrove roots (preventing anaerobic conditions) and soil microbes. Furthermore, they play an important role in the recycling of nutrients and, thus, they have a significant effect on microbial communities (Zeil et al. 2006). In addition, when fiddler crabs filter mangrove soil, they mobilized bacterial communities in the sediments, which means, they have an indirect role in the recycling of nutrients.

Fiddler crabs (Ocypodidae: genus *Uca*) are small crustaceans (can reach a body size of up to 5 cm) that inhabit sandy and muddy intertidal areas of estuaries. They are most often found in soft sand or mud near or around the edges of shallow salt marshes and mangrove sediments. *Uca* species are dimorphic animals: males have one clamp much larger than the other while in females both clamps are small. The major clamp plays an important role in agonistic behaviour and in courtship (Crane, 1975). Most of their time is spent on feeding, while their leftover time is dedicated to burrow maintenance, social interactions, and predator avoidance (Crane, 1975). This means that the most important structure for fiddler crabs are their own burrows, which provide protection against predators, refuge for molting and for females while incubating their eggs, and protection against desiccation when the surface does not provide water, which is needed for respiration and feeding, among others (Hemmi et al. 2003). Fiddler crabs may dig more burrows than they require for their protective and physiological needs, especially when food resources are low (Genoni, 1991), which means that they are sensitive to food availability, creating burrows

faster and with less depth. Their body size is also correlated to food availability (Castiglioni et al. 2004).

The genus *Uca* is divided approximately into 100 species, which thrive around the world in the muddier parts of the tropics and subtropics; and have even reached New England and Japan (Crane, 1975, Beinlich & von Hagen 2006; Ng et al. 2008) [for global distribution see Appendix A]. The species *Uca rapax* (Smith, 1870) is one of the most abundant of the genus inhabiting the muddy sand of subtropical mangroves, specific in tropical and subtropical western Atlantic (Crane, 1975; Castiglioni et al. 2004) [Appendix. B]. This particular species belongs to the subgenus of *Minuca* and the subspecies *rapax* (Figure no. 1.1). Some of the morphological characteristics of *Uca rapax*, according to Crane, (1975) are: male differs in the absence of pile in lower margins of merus, carpus, and manus; while females differs in the presence of a distinct tubercle on the gonopore and the absence of pile on lower margins of ambulatory meri [Appendix C].

In summary, the primary ecological role of *Uca rapax* is recycling of organic matter (Castiglioni et al. 2004) by burrowing deep into the mud, and also creating a maze of tunnels, which aerate the estuarine soil and promote aerobic conditions for the soil. They contribute to the maintenance of the productivity and nutrient fluxes of ecosystems (Kristensen et. al. 2008), especially mangrove forests in tropical and subtropical regions of the planet.

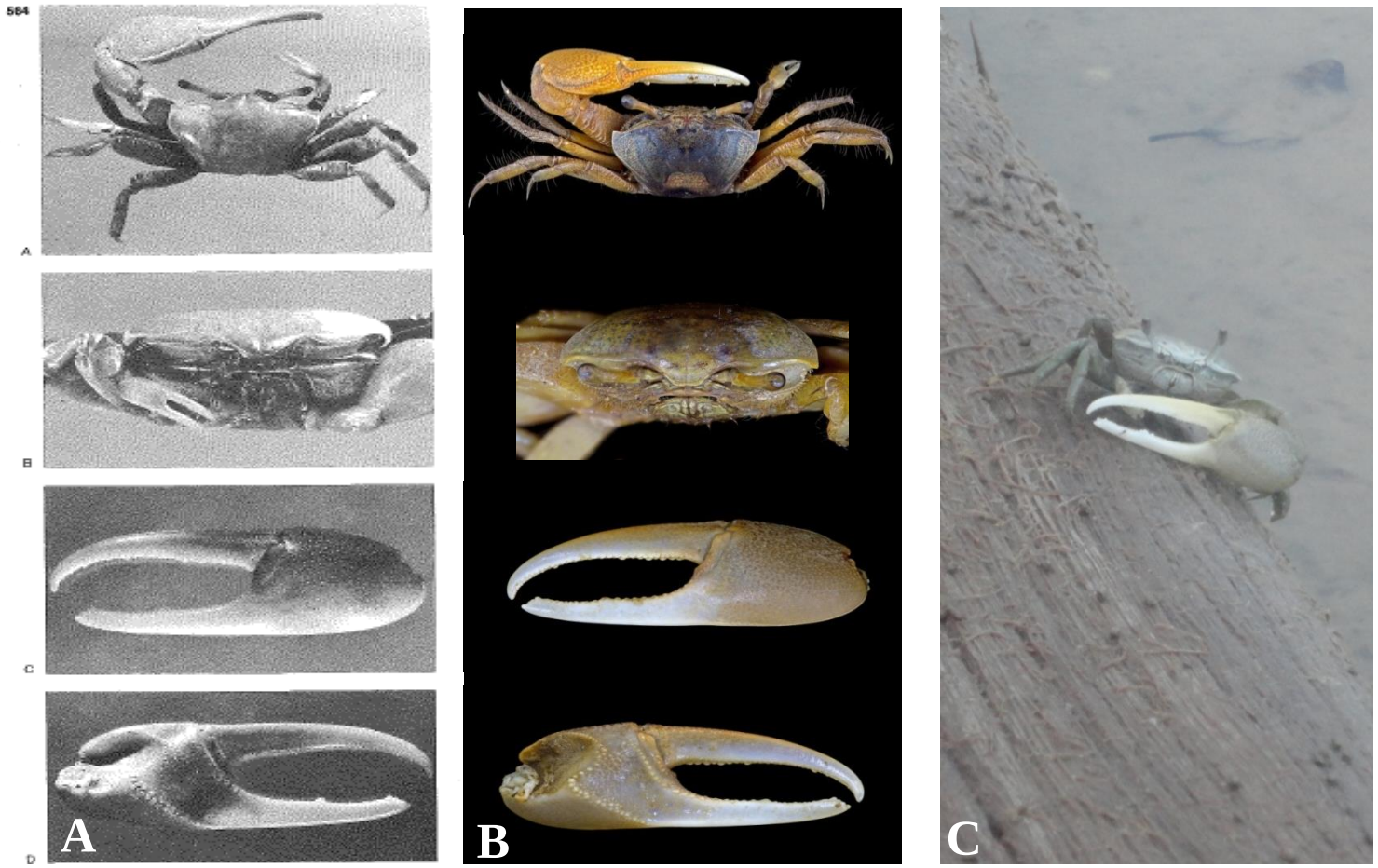


Figure no. 1.1: Fiddler crab: *Uca rapax*. (A) *Uca rapax* described and reproduced from Crane (1975). (B-C) *Uca rapax* collected from "Bosque Estatal de Boquerón" (Guaniquilla mangrove).

1.3 Goals

1.3.1 Hypotheses

1. There is specific microflora associated with the hindgut of *Uca rapax,* which is different from the microflora found in the mangrove sediments.
2. The specific microflora associated with the hindgut of *Uca rapax* is capable to degrade cellulose compounds.

1.3.2 Objectives

1. The principal goal of this study is to identify and characterize the bacteria associated with the hindgut of the fiddler crab, *Uca rapax* (Smith, 1870), from Guaniquilla mangrove in Boquerón, Cabo Rojo, Puerto Rico.

    a) Characterize the diversity of bacteria found in the mangrove sediment.

    b) Characterize the diversity of bacteria associated with the hindgut of *Uca rapax*.

    c) Compare communities during the wet (rainy) and the dry seasons.

    d) Compare mangrove bacteria and hindgut microflora communities.

2. Characterize cellulose degradation of gut microflora.

    a) Study cellulose degradation of hindgut bacteria through cellulolytic enzyme assays.

Chapter 2: Diversity of bacteria associated with mangrove soil

2.1 Introduction

Mangroves are coastal, biologically important and productive ecosystems in tropical and sub-tropical regions. They are complex and dynamic environments varying in salinity, water levels and nutrients availability. Microbes play an important role in governing the biogeochemical cycles of any ecosystem. Mangroves are very rich in organic matter, allowing microbes, especially bacteria, to be active participants of this ecosystem (Saleem-Khan et al., 2009). According to Santos et al. (2010) mangrove bacteria are directly involved in the transformation of nutrients, photosynthesis, nitrogen fixation, methanogenesis, phosphate solubility, sulfate reduction and production of other substances, among others. There is particular interest in bacteria from the phylum Proteobacteria, which are represented by different orders involved in cycles of mangrove ecosystems, such as Rhizobiales, Campylobacterales, Methylococcales and Vibrionales. Species of *Vibrio* have been isolated from salt marsh and mangrove rhizospheres that might be involved in nitrogen fixation, which is linked towards successful development of plants in such environments (Gomes et al. 2011). Bacteria distribution depends on changes in water, temperature, depth of soil, salinity and other physicochemical parameters (Alavandi, 1990; Saleem-Khan et al. 2009). In this section, we analyzed soil samples from the entrance of the fiddler crab burrow, comparing bacteria communities from both wet and dry seasons.

2.2 Materials and Methods

* All the procedures described herein were implemented on both, mangrove soil and *Uca rapax*'s hindgut samples.

2.2.1 Sampling area

We took samples of mangrove sediments and fiddler crabs (*Uca rapax*) from the south-western region of Puerto Rico, specifically in the mangrove forest in the Guaniquilla area of the "Bosque Estatal de Boquerón" in Cabo Rojo (18°1'41.16'' N and 67°10'30.72 W) [Appendix D]. Boquerón sector presents an average annual temperature which ranges from 25.1° C to 27.0° C and its average annual rainfall varies between 730 mm and 860 mm, as it is located in the arid climate region of the island. Dry season usually occurs among the months of January and April, while the highest precipitation occurs August through November (DRNA, 2008). The combination of high temperatures and low precipitation causes a high rate of evaporation, which is an important climatic factor in determining the establishment of different types of natural systems. Partially, these conditions may explain why these ecosystems are so fragile and of slow recovery.

Weekly precipitation corresponding to the wet season (August 2011) was on average 5.59 mm of pluviometric water; meanwhile, in the dry season (March 2012) there was only 0.9 mm of pluviometric water reported. We also performed a pre-sampling (November 2010), during which an average precipitation of 2.03 mm of pluviometric water was reported (http://www.wunderground.com). This information during samplings can help explain the dynamics of bacterial communities in different seasons (wet vs. dry).

2.2.2 Soil sampling

During the wet and the dry seasons (on the same dates in which crabs were collected), six mangrove soil samples were taken from the entrance of the fiddler crab burrows using sterilized centrifuge tubes. One gram of mangrove soil was dissolved in 100 mL of sterile distilled water. A successive serial dilution was prepared to a final dilution of 10^{-4} and 200 µL of the suspension were inoculated in triplicate to different culture media.

2.2.3 Bacteria pure culture isolation

Mangrove soil dilutions were inoculated separately into four different culture media with different nutritional properties in order to obtain greater bacterial diversity. The four media were: Chitin [$ZnSO_4$ • $7H_2O$ (0.000075%w/v); $MnCl_2$ • $4H_2O$ (0.000075%w/v); $FeSO_4$ • $7H_2O$ (0.00075%w/v); KH_2PO_4 (0.0275%w/v); $MgSO_4$ • $7H_2O$ (0.0375%w/v); K_2HPO_4 (0.0575%w/v); Chitin (0.3%w/v) and agar (1.5%w/v)]; Lenox broth (LB) (Sigma Chemical Co.); Marine agar (HiMedia Laboratories) and Mangrove soil agar. The mangrove soil extract was prepared according to Zuchi (2009), with 200 g of surface mangrove soil boiled in 1L of distilled water for 5 min. The boiled soil was cooled for 15 min, and each liter of the soil medium was prepared with 100 mL of soil supernatant, glucose (0.4%w/v), K_2HPO_4 (0.5% w/v), sodium nitrate (0.1%w/v) and agar (1.5%w/v), and the pH was adjusted to 7. All media were sterilized and complemented with the antifungals Nystatin (0.02 g/ml of DSMO) and Cyclohexamide (0.05 g/L).

We spreaded the bacterial suspension on plates, which were incubated at 25-30°C during four days up to three weeks. Several transfers of the cultures were made to the respective medium with antifungals in order to obtain pure colonies. All samples were preliminary classified using morphological characteristics and Gram staining (Brenner et al., 2005).

2.2.4 DNA extraction, 16S rDNA gene amplification and sequencing

For comparison purposes, DNA extraction and 16S rDNA gene amplification from the isolated mangrove soil bacteria were performed. We extracted the DNA from all isolates from pure cultures using the CTAB chemical method. This method consists of a lysis in the presence of the detergent Cetyl trimethyl-ammonium bromide (CTAB). We also used lysozyme, which can help in disrupting the murein bacterial cell wall. Physical methods, grinding and sonication, were also used to aid cell wall breakage. Samples were extracted with chloroform, transferred to precipitation with 100% isopropanol and washed twice with 70% ethanol. The final step was to resuspend the samples in TE 1/10 (10 mM Tris-HCL and 0.1mM EDTA) buffer and stored at -20°C.

PCR amplification of 16S rDNA gene reaction for a final volume of 25 µL was carried out using 1:50 dilution of genomic DNA as template, 0.8x PCR buffer, 3 nM $MgCl_2$, 0.6 mM of each primer, 0.16 mM dNTPs and 5U Taq polymerase per reaction. We used the universal bacterial primers 27F [5′-AGAGTTTGATCCTGGCTCAG-3′] and 1492R [5′-GGTTACCTTGTTACGACTT-3′] (Lane, 1991) and the following thermal parameters: 95°C: 3 min, 30 x (95°C: 45 sec, 52°C: 45 sec, 72°C: 1.5 sec) and 72°C: 10 min for the amplification.

Sanger sequencing was implemented with the same primers at the High-Throughput Genomics Unit of the University of Washington, Seattle, WA.

2.2.5 Diversity of mangrove soil bacteria: Data Analysis

2.2.5.1 Diversity Indices

Microbial community biodiversity in mangrove soil was evaluated through Simpson and Shannon-Wiener indices. We used the Simpson index (D) as an estimation of dominance, which represents the probability that two individuals randomly selected from a sample will belong to the same (D) or different (S) species. Where; n = the total number of organisms of each individual species; N = the total number of organisms of all species.

Simpson Index of Dominance (D):	Simpson Index of Diversity (S):
$$D = \sum \frac{n(n-1)}{N(N-1)}$$	$$S = 1 - D$$

We also used the Shannon-Wiener index (H') as an estimation of evenness. Where: n_i = number of individuals or amount of each species; N = total number of individuals (or amount) for the site, and ln = the natural logarithm of the number.

Shannon-Wiener (H'):

$$H' = -\sum \left[\left(\frac{n}{N}\right) x\ ln\left(\frac{n}{N}\right)\right]$$

To calculate the biodiversity indices of Simpson and Shannon-Wiener we used the free software application EstimateS 8® (Colwell, 2009) that computes a variety of biodiversity

functions, estimators, and indices based on biotic sampling data. Is important to mention, that the Simpson index's output result obtained from EstimateS 8® (Colwell, 2009) is specifically the Simpson Reciprocal Index, which means: $\frac{1}{D}$.

2.2.5.2 Bacterial accumulation curve and comparison of microbial communities

The accumulation curve was performed to calculate the expected richness of species by the number of samples or total individuals. It is important to study whether the estimated richness calculated is representative of the real richness that could be found in the environment; for example, within the hindgut of fiddler crabs or in mangrove soil. We also used the free software application EstimateS 8® (Colwell, 2009) to obtain the expected species accumulation (Mao Tau values) and number of individuals collected and, then, plotted the accumulation curve using Excel® (Microsoft® Excel®, 2010) program.

The microbial communities between seasons (wet and dry) were compared to determine if there were significant differences. We used the phylogenetic method implemented in UniFrac® (Lozupone et al. 2005) for comparison of the microbial communities, specifically the UniFrac Significance analysis with 100 permutations.

2.2.5.3 Sequence Analysis

Sequences of the 16S rDNA gene were edited and analyzed using Sequencer 4.8® (Gene Codes, Ann Arbor, MI). Then, GenBank® (Benson et al. 2013) searches with BLASTn® (Altschul et al. 1990) were performed to identify the closest available sequences. Phylogenetic analysis was conducted in MEGA5® (Molecular Evolutionary Genetics Analysis) (Tamura et al. 2011), which is an integrated tool for conducting sequence analysis. This program permits

manual and automatic sequence alignment using ClustalX® (Tamura et al. 2011). Phylogenetic trees were inferred also in MEGA 5® (Tamura et al. 2011) by Neighbor-Joining, which establishes the percent sequence difference for all pairwise combinations to infer relationships between taxa. Branch support was evaluated by the bootstrap method after 5,000 replications.

2.3 Results

2.3.1 Bacterial communities associated with mangrove soil during the wet and the dry seasons

Using culture-dependent methods we identified and characterized a total of 233 bacteria strains associated with mangrove soil in both seasons [Appendices: E-F]. In the wet season, we identified 106 bacteria representing 25 genera (Table no. 2.1) distributed in: Actinobacteria (54%), Firmicutes (33%), Proteobacteria (12%) and Bacteroidetes (1%). The three most abundant genera were *Bacillus* (22%), *Microbacterium* (15%) and *Gordonia* (10%). In the dry season, we identified 127 bacteria in 22 genera (Table no. 2.1) distributed in Firmicutes (74%), Actinobacteria (18%) and Proteobacteria (8%). The three most abundant genera were *Bacillus* (48%), *Halobacillus* (16%) and *Streptomyces* (6%). Different culture media with different nutritional properties allowed to isolate selective bacterial genera. Chitin agar, Marine agar and Mangrove soil showed a greater richness of isolated genera in both seasons (Figure no. 2.1 and no.2.2). The genus *Bacillus* (peach color) was recovered from all culture media, although in different proportions. In addition, this genus was the most abundant overall in both seasons, representing 36% year-round global of all isolates.

Table no. 2.1: Bacterial genera isolated from mangrove soil in the wet and the dry seasons.

Season	Genera of bacteria
Wet	*Bacillus, Microbacterium, Gordonia, Isoptericola, Staphylococcus, Lysinibacillus, Mycobacterium, Oceanimonas, Rhodococcus, Serinicoccus, Halomonas, Microbulbifer, Cellulosimicrobium, Sphingomonas, Cellulomonas, Aeromicrobium, Agrococcus, Algoriphagus, Brevibacillus, Curtobacterium, Demequina, Nitratireductor, Nocardia, Novosphingobium* and *Planococcus*
Dry	*Bacillus, Halobacillus, Streptomyces, Microbulbifer, Paenibacillus, Lysinibacillus, Isoptericola, Arthrobacter, Marinobacter, Microbacterium, Micromonospora, Terribacillus, Brevibacillus, Gordonia, Mycobacterium, Nocardia, Nocardioides, Novosphingobium, Rhodococcus, Ruegeria, Corynebacterium* and *Kocuria*

In summary, we isolated 36 bacterial genera from six mangrove soil samples located at the entrance of the fiddler crab burrows (Figure no. 2.3). There were 11 genera that were present in both seasons (*Bacillus* sp., *Microbacterium* sp., *Isoptericola* sp., *Gordonia* sp., *Lysinibacillus* sp., *Microbulbifer* sp., *Mycobacterium* sp., *Rhodococcus* sp., *Brevibacillus* sp., *Nocardia* sp. and *Novosphingobium* sp.) and the rest of genera were present only at the wet season or the dry season (Figure no. 2.3).

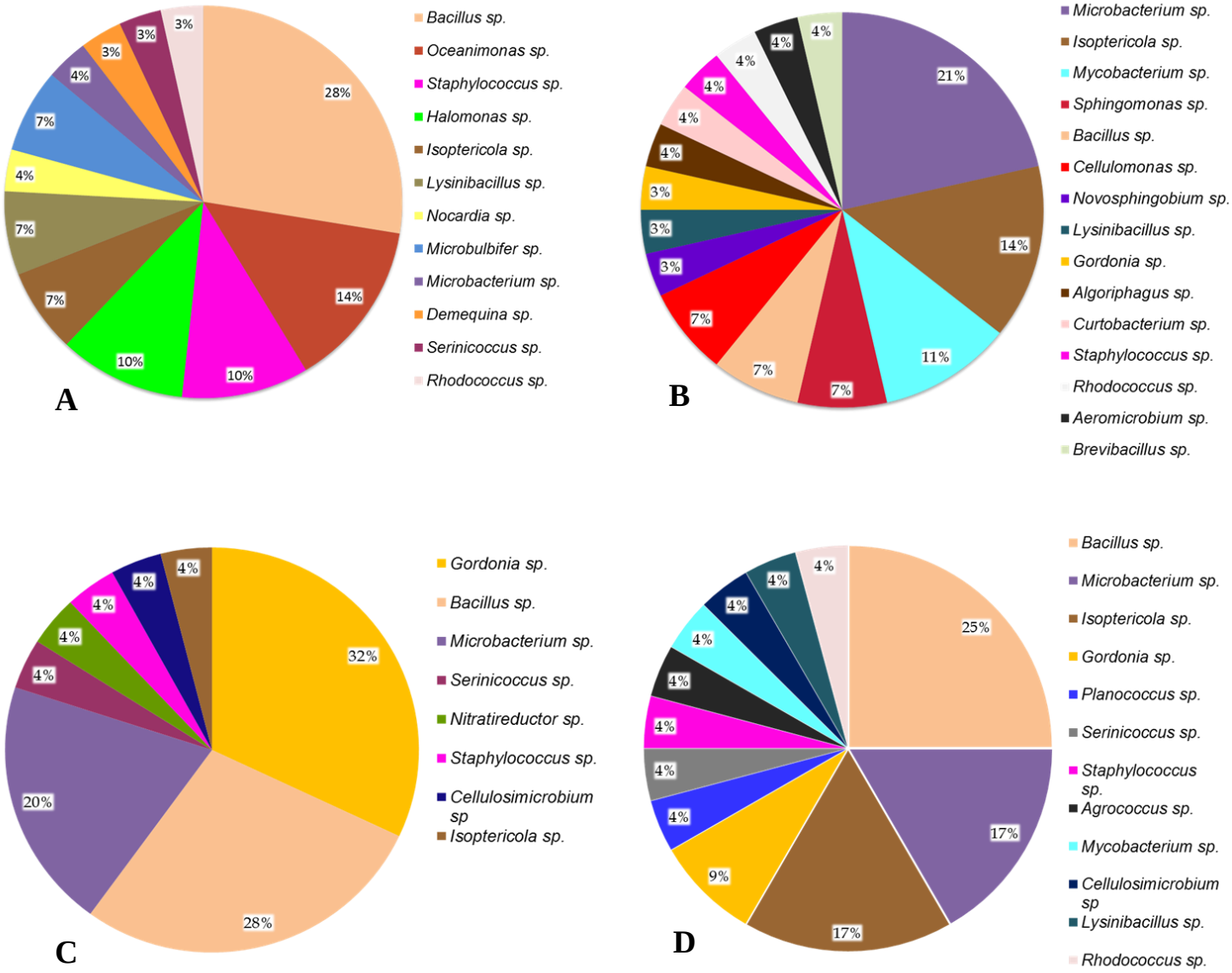


Figure no. 2.1: Genera of bacteria isolated from mangrove soil in the wet season. (A) Marine agar, (B) Chitin agar, (C) LB agar, and (D) Mangrove soil agar.

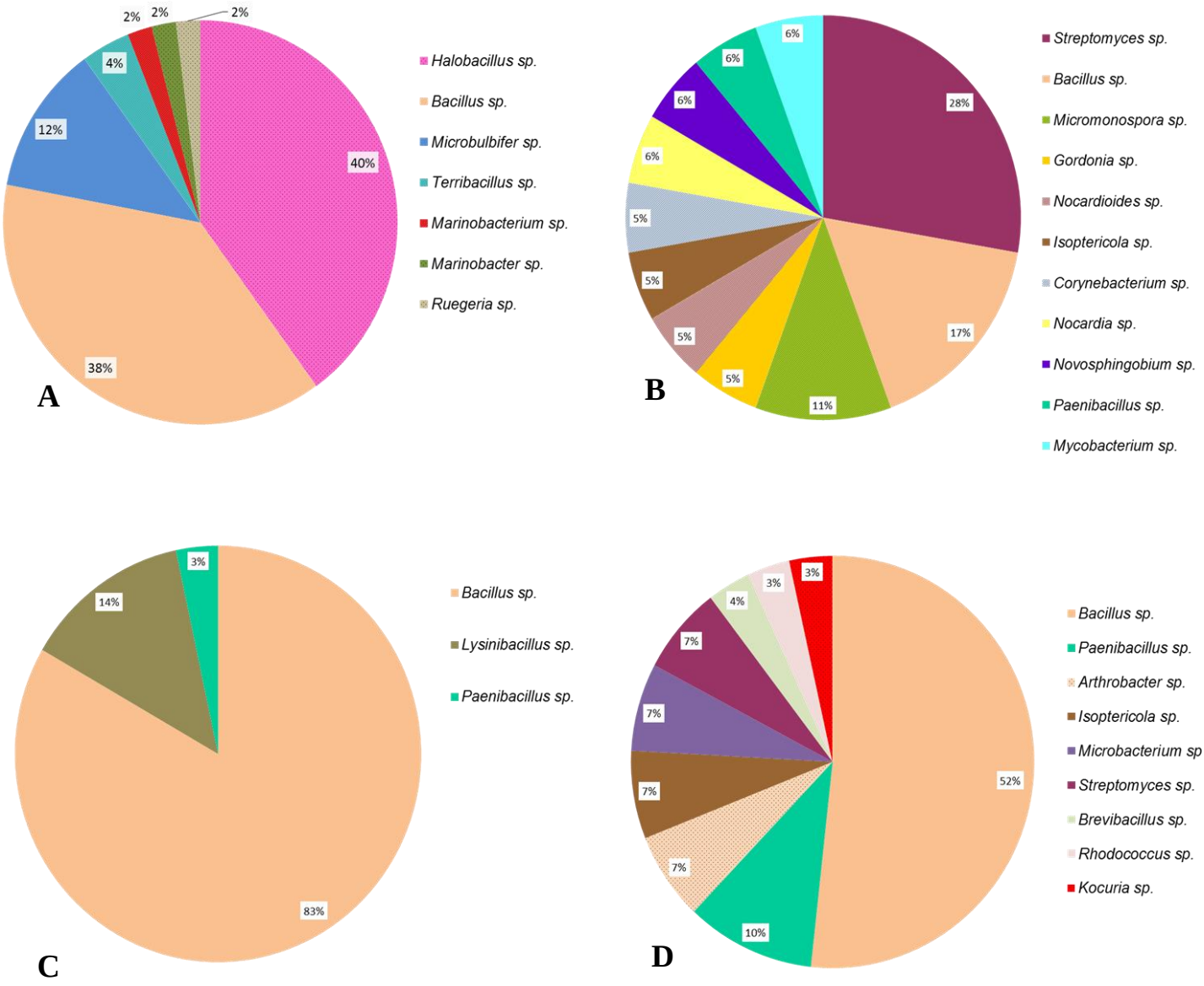


Figure no. 2.2: Genera of bacteria isolated from mangrove soil in the dry season. (A) Marine agar, (B) Chitin agar, (C) LB agar, and (D) Mangrove soil agar.

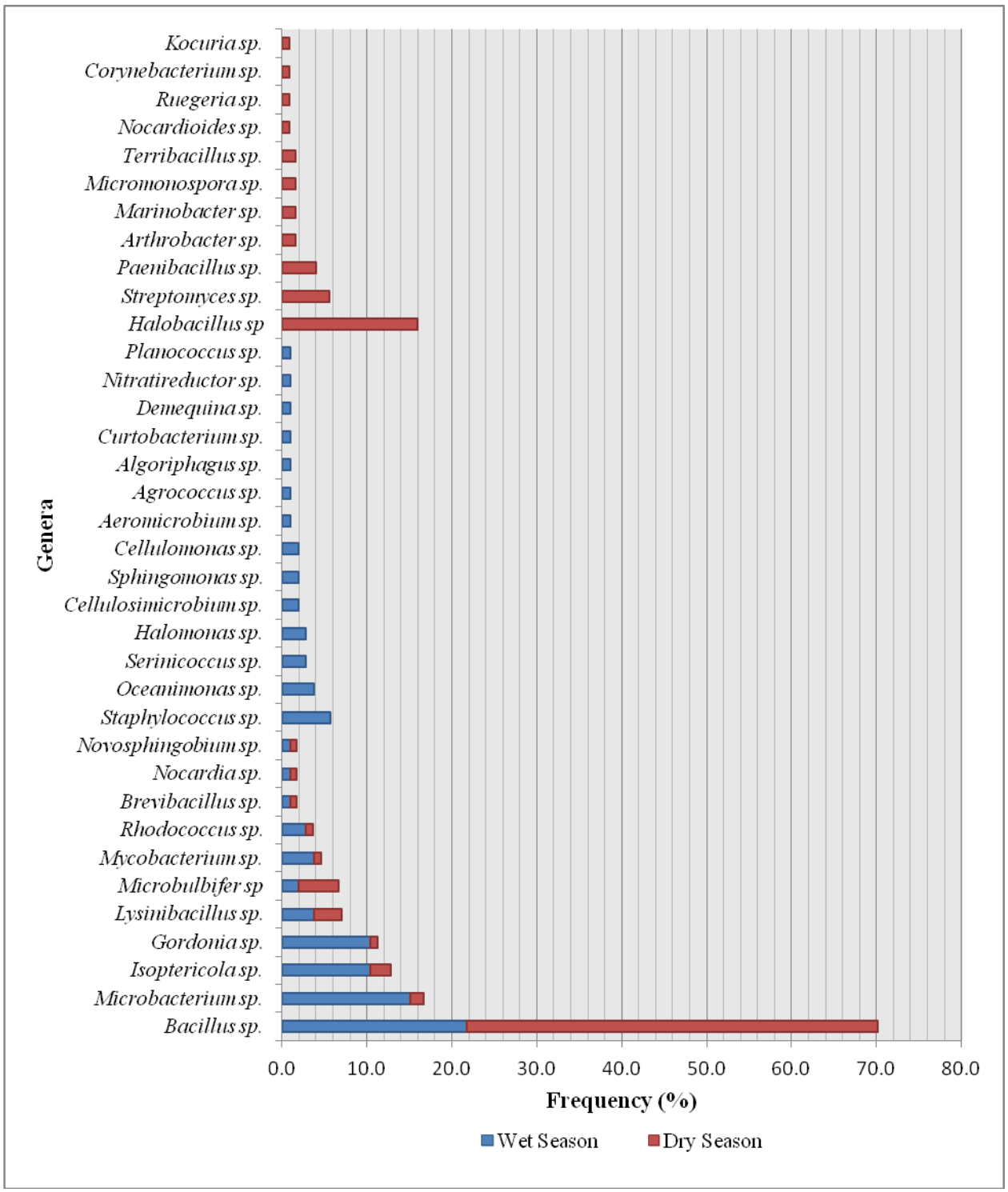


Figure no. 2.3: Bacterial genera abundance isolated from mangrove soil in the wet and the dry seasons.

2.3.2 Diversity indices and bacterial accumulation curve of mangrove soil bacteria.

To describe the biodiversity of the cultivable mangrove soil bacteria we calculated diversity indices (Simpson's Index and Shannon-Weiner's Index) and also we created an accumulation curve of the 233 strains isolated from the wet and the dry seasons. The term "Simpson's index" can refer to three related indices: Simpson's index of dominance, Simpson's index of diversity or Simpson's reciprocal index. Simpson's index of dominance (D) measures the probability that two individuals randomly selected from a sample will belong to the same species (genera) or different species (Simpson's index of diversity [S= D-1]). The Simpson's index of diversity value ranges is 0 and 1; in which 0 represents low diversity and 1 represents high diversity (Simpson, 1949). All the mangrove soil bacteria (36 genera from 233 strains) presented a Simpson's index of diversity value of 0.8569 indicating a high diversity and low dominance in the community (Table no. 2.2).

We also calculated Shannon-Weiner's index (H'), which measures the average degree of uncertainty (synonymous with diversity) of predicting the species of a given individual picked at random from a community (Brown et al., 2001); the index varies from a value of 0 for communities with only a single species to high values for communities having many species. The Shannon-Weiner's index value was 2.4767, indicating that the community is composed of many taxa (Table no. 2.2).

In order to evaluate the sampling effort in this study, we created an accumulation curve (Figure no. 2.4) with the strains isolated. The results suggest more sampling is needed because the curve does not reach an asymptote.

Table no. 2.2: Diversity indices of mangrove soil bacteria.

Diversity Indices		
Whole mangrove soil	-Simpson's Index of Dominance (D)	0.1431
	-Simpson's Index of Diversity (S=1-D)	**0.8569**
	-Simpson's Reciprocal Index (1/D)	6.9881
	-Shannon-Weiner's Index (H')	**2.4767**
Wet mangrove soil	- Simpson's Index of Diversity (S=1-D)	0.8620
	-Shannon-Weiner's Index (H')	2.3500
Dry mangrove soil	- Simpson's Index of Diversity (S=1-D)	0.8517
	-Shannon-Weiner's Index (H')	2.6000

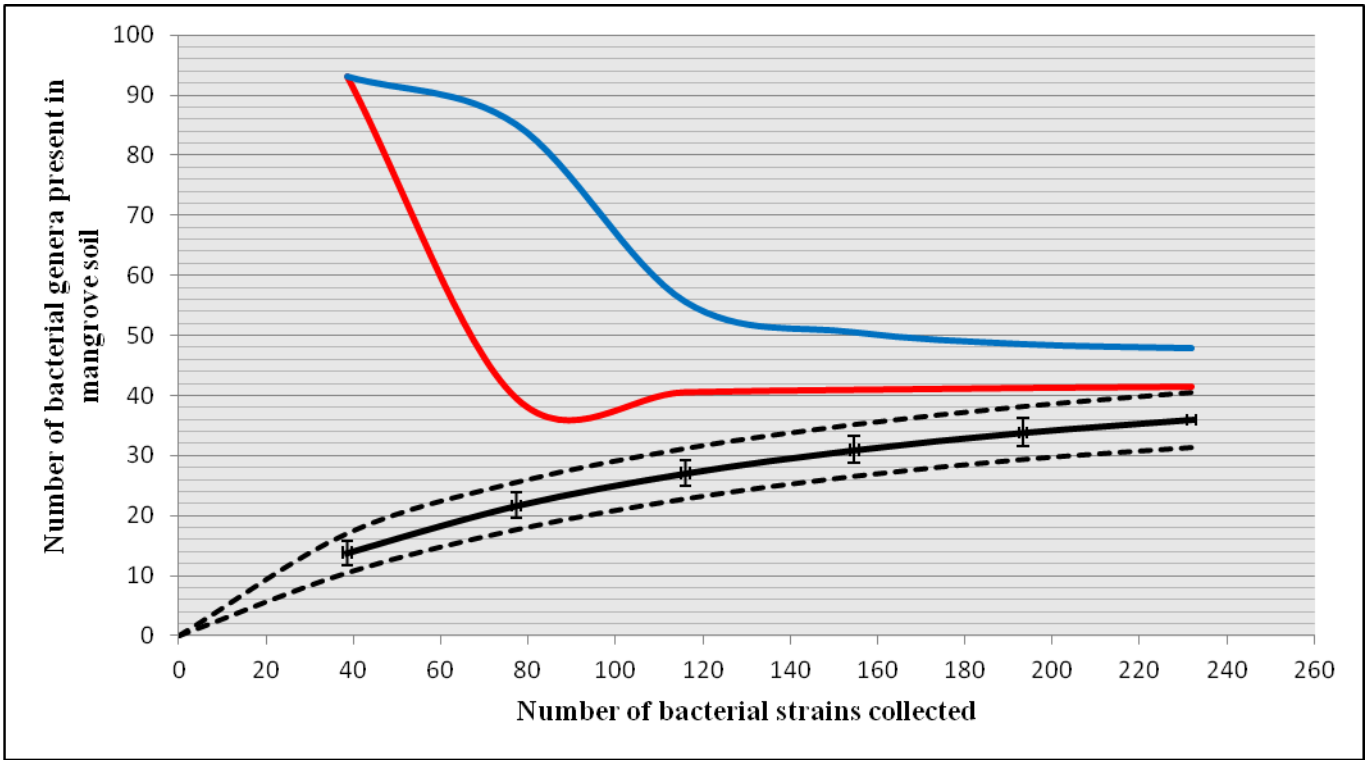


Figure no. 2.4: Sample-based bacterial accumulation curve displaying number of bacterial strains collected against number of identified bacterial genera (Mao Tau [Sobs] values) present in mangrove soil from the entrance of the fiddler crab burrows. Dashes lines represent a 95% of confidence intervals. And the corresponding richness estimators: Chao 2 Mean (red line) and ICE (incidence-based coverage estimator) Mean (blue line).

2.3.3 Comparison between mangrove soil bacteria in the wet and the dry seasons

All mangrove soil bacteria were compared between the wet season (MD_W) and the dry season (MD_D) using Unifrac® (Lozupone et al. 2005). The results indicated that there are significant differences between seasons in terms of bacterial composition (Table no. 2.2).

Table no. 2.3: Matrix showing p-values of multiple comparisons of mangrove soil bacteria in the wet and the dry seasons using Bonferroni corrected data. Colors indicate the significant difference on a scale defined by Unifrac®.

	MD_D	MD_W
MD_D		<=0.01
MD_W		

Color	Description
	(< 0.001) Highly significant
	(0.001-0.01) Significant
	(0.01-0.05) Marginally significant
	(0.05-0.1) Suggestive
	(> 0.1) Not significant

2.3.4 Phylogenetic analysis of mangrove soil bacteria

We created a 16S rDNA phylogenetic tree comparing all the mangrove soil bacteria of both seasons (Figure no. 2.5). The principal objectives are to determine the phylotypes represented in soil, to compare wet and the dry season's taxa, and to detect season-specific clades. The phylotypes were Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes (Figure no. 2.5, clockwise). Our results indicate there are distinctive clades formed only by sequences from the same season, which supports the differences in bacterial composition between seasons detected in other analyses.

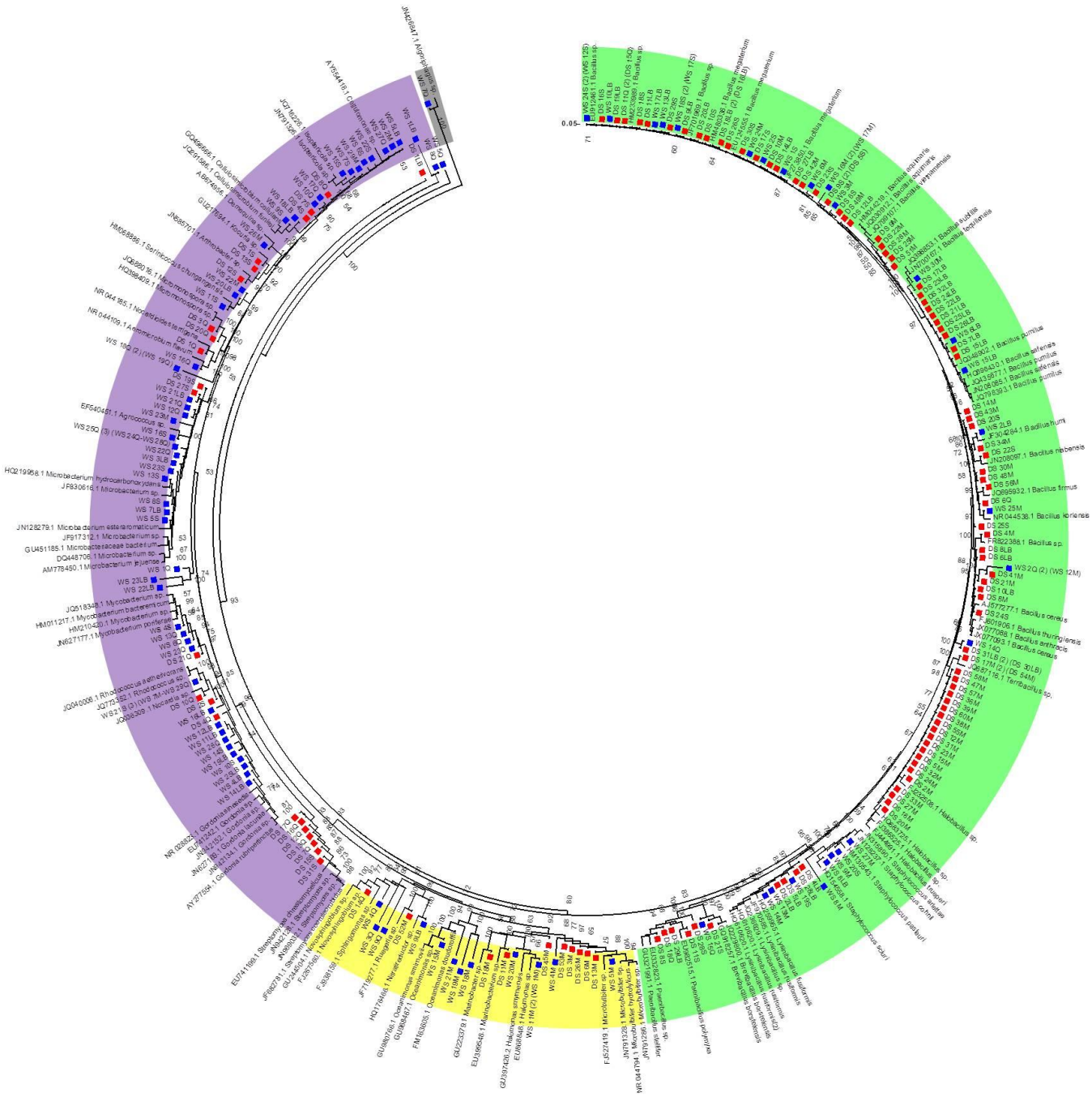


Figure no. 2.5: Phylogenetic Neighbor-joining tree based of 16S rDNA gene sequences from isolated mangrove soil bacteria (sequences from the wet season are blue colored and sequences from the dry season are red colored) and closely related species found in GenBank®. The phylotypes were Firmicutes (green shade), Proteobacteria (yellow shade), Actinobacteria (purple shade) and Bacteroidetes (grey shade). The phylogeny is based on partial 16S rDNA sequences of approximately 1,000bp. The numbers at the nodes indicate bootstrap support values (>50%) based on 5,000 pseudoreplicates. The scale bar corresponds to 0.05 substitutions per site.

2.4 Discussion and Conclusion

The microbial community associated with mangrove soil plays a critical role in cycling of nutrients and thus control the chemical environment of the mangrove ecosystem. Bacteria communities change according to water, temperature, salinity, depth of soil and other physicochemical parameters. We expected the diversity of bacteria to change over time and specifically to differ between seasons due to greater average precipitation in the wet season (August 2011). Nonetheless, our results presented similar diversity in both seasons (WS = 25 genera, 106 strains; DS = 22 genera, 127 strains) although the relative abundance changed for some groups (i.e. WS = 54% Actinobacteria, 33% Firmicutes vs. DS = 74% Firmicutes, 18% Actinobacteria). Actinobacteria and Firmicutes are typically dominant in soils because they can resist adverse conditions, especially desiccation through endospore formation. As a high stress environment, these bacteria possibly form endospores to survive and; when placed in culture media, they will outcompete and grow faster than other bacteria (gram negative) that do not have these stress adaptations. This effect could explain why the Firmicutes were so abundant in this study. The actinobacteria genera *Isoptericola*, *Micrococcus*, *Microbacterium*, *Nocardia*, *Rhodococcus* and *Streptomyces* were also reported from mangrove soils in China (Hong et al. 2009); and *Microbacterium* and *Brevibacillus* were also reported from a mangrove sediment in Brazil (Dias et al. 2009). Several species of *Streptomyces* have been reported from different mangrove environments in India (Thatoi et al. 2013). In the Philippines, cellulose-producing bacteria belonging to Firmicutes group, such as *Bacillus cereus* and *Bacillus pumilus*, among others, have been reported from mangrove soils (Tabao et al. 2010). In contrast to other reports (Holguin et al. 1992; Liao et al. 2007; Gomes et al. 2011), Proteobacteria was not the predominant group and, more specifically, *Vibrio* was not isolated from mangrove soil in

Boquerón. The microbial community in mangrove soil from Boquerón in both seasons was dominated by three genera: *Bacillus, Microbacterium* and *Isoptericola*, which composed approximately 90% of the sampled diversity out of 36 genera recovered. All other 33 genera represented less than 10% of the community described. The culture medium is an important factor in isolating diverse bacteria, being Marine agar and Mangrove soil agar the media where greater numbers of genera were isolated. These media have the nutritional properties that most closely resemble the mangrove ecosystem, especially in regard to the salinity, hence allowing some bacteria to grow under these conditions.

The biodiversity indices of Simpson (0.8569) and Shannon-Weiner (2.4767) indicated a high diversity of bacterial genera in the mangrove sediments based on culture dependent methods. Nonetheless, the bacterial accumulation curve suggests more sampling effort is needed to obtain the real diversity that can be found in the mangrove sediments when we only consider cultivable bacteria. According to the richness estimator, Chao 2, the accumulation curve represents an 89% of the 0.1% of cultivable diversity found in the mangrove sediments. These results are due to the small inocula spread on media or due to the limitation of culture media, which represent the mangrove environment. In addition, the matrix of multiple comparisons by UniFrac® and the phylogenetic tree indicated that there are significant differences between the wet and the dry seasons. We can assume two possible explanations for the significant differences between seasons: moisture and litterfall variability. Actinobacteria and Firmicutes were the predominant groups in both seasons. In the dry season these groups are expected to dominate due to their thick peptidoglycan layer in their cell walls, which makes them more resistant to desiccation stress than Gram-negative bacteria (Schimel et al. 2007; Lennon et al. 2012).

The Guaniquilla mangrove forest has four mangrove species, but *Rhizophora mangle* and *Laguncularia racemosa* are the most common and dominant. The litterfall comprised by the leaves of these two mangrove species might also change the microbial community in the mangrove sediments. Lugo et al. (2007) measured the litterfall rate of these mangrove species (*Rhizophora mangle* and *Laguncularia racemosa*) from mangrove forest in Jobos Bay, Puerto Rico, during the year 1986. Both mangrove species have a greater rate of litterfall in the months of March and April and then decreased for the rest of the year. If we correlate their results with ours, we would expect a greater richness and abundance of mangrove bacteria (at the dry season) according to the raise in the rate of leaf fall, which results in more organic matter. Our results support these ideas, indicating a greater abundance of mangrove bacteria in the dry season (127 strains) when compared to the wet season (106 strains), but also less diverse in terms of genera (22 genera vs. 25 genera from the wet season). This implies that even though there is an increase of nutrients, only some bacteria genera are capable to tolerate specific physico-chemical conditions such as salinity and desiccation.

In order to understand the dynamics of microbial populations according to biotic or abiotic factors, we can assume that litterfall (increased nutrients) might be dictating strongly the abundance within microbial communities, more than the abiotic effect of water availability between seasons, assuming that bacteria in these ecosystems must resist times of desiccation.

We recommend studying the microbial diversity of mangrove bacteria based on depth of soil (Saleem-Khan et al. 2009) by culture-independent methods and to also analyze the community of anaerobic bacteria present in the mangrove ecosystems, which were not considered in this study.

Chapter 3: Diversity of bacteria associated with the hindgut of *Uca rapax*

3.1 Introduction

Fiddler crabs are deposit-feeders; detritus forms part of their diet, though filtered sand may contain particles of algae, bacteria, ciliates, archaea and fungi (Robertson et al. 1982). Moreover, they filter small particles in their mouthparts and transport cellulosic material, algae, bacteria, and fungi to the gut for digestion. It is suggested that some soil-derived bacteria may be attached to the intestine forming a symbiotic association over time. According to Gulmann (2004), this symbiotic microbiota may assist in degrading the lignocellulose rich fraction of the diet by depolymerizing cellulose and fermenting the resulting carbohydrates to short-chain fatty acids.

There is little scientific evidence for this nutritional symbiosis between bacteria and mangrove fiddler crabs, but it is clear that such partnership would lead to many benefits. Gut microbes may contribute to the host nutritional fitness by increasing the extracellular enzymes to degraded organic matter during digestion, supplying vitamins, favourably altering the chemistry of the gut environment and/or preventing the proliferation of pathogenic bacteria (Harris 1993). Furthermore, the host may aid resident gut microbes by providing nutrients, a relatively constant environment and protection from predators (Plante et al. 1990). Even so, this association may not occur throughout all the digestive system; different gut sections may have a particular chemical environment.

The gut of crustaceans is divided in three principal regions with specific functions (Dall et al. 1983). The foregut, whose function is to transmit the secretion of the digestive gland, filters

the food after passing it backward through the gastric mills. The midgut, which serves as dual role of enzyme secretion for digestions of proteins, lipids and some carbohydrates, and absorption of digested food. And finally, the hindgut whose principal function is defecation and also, the anal drinking of water, which pumps the water from the anus into the gut. This implies that, when microbial communities in the hindgut secrete enzymes to degrade the carbohydrates, the nutrients can move out to the midgut for absorption.

Gulmann (2004) reported that the hindgut of the marsh fiddler crab *Uca pugnax* harbors three times more bacteria compared to other gut sections, such as stomach or midgut. According to Lau et al. (2002) the main function of the hindgut is to store fecal matter and is where the host's defenses against microbes are weakest. Hindgut bacteria do not compete with the host for nutrients because absorption mainly occurs in the midgut. Many gut-associated bacteria produce enzymes that the invertebrate host cannot (Harris, 1993). The most commonly reported genera for aquatic invertebrate gut microbiota include: *Vibrio*, *Pseudomonas*, *Flavobacterium*, *Micrococcus* and *Aeromonas* (Harris, 1993). Although, it is possible that these gut microbial communities are either ingested transient microbes or symbionts, and both can provide nutritional benefits to host's fitness. The roles of the gut microbiota in the physiology of the invertebrate host and its contribution to nutrient recycling in the mangrove ecosystem have not been well established.

3.2 Materials and Methods

3.2.1 Fiddler crab, *Uca rapax* sampling

In the Guaniquilla mangrove region of the "Bosque Estatal de Boquerón" in Cabo Rojo [Appendix D], there are only five species of the genus *Uca*: *U. rapax*, *U. burgersi*, *U. thayeri*, *U. vocator* and *U. major* (Rivera et al. 1982). During the wet and the dry seasons we collected by hand a total of nine male crabs of the species *Uca rapax.*

The crabs were surface-sterilized with hypochlorite (6%) and ethanol 70% to prevent contamination of bacteria from the external body surface, when removing the hindgut. The fiddler crabs were dissected by removing the abdomen and pulling the hindgut with sterile forceps. The hindgut was immediately placed into sterile microfuge tubes with sterile deionized and distilled water (1 mL) and macerated by mechanical methods (sonication and vortexing) to dislodge the bacteria. After a series of dilutions (10^{-4}), 200 µL of the suspension were inoculated in triplicate, into different culture media.

Details of sampling area were described previously in Chapter 2 (page 11).

3.2.2 Bacteria pure culture isolation

Suspensions of bacteria from the hindgut microflora were inoculated separately into four different culture media with distinctive nutritional properties in order to isolate more bacterial diversity. The four media were: Chitin agar, Lenox broth (LB) agar, Marine agar and Mangrove soil agar. All media were sterilized and complemented with the antifungals Nystatin (0.02 g/ml of DSMO) and Cyclohexamide (0.05 g/L). We spread the suspension in the plates, which were

incubated at 25-30°C during four days to up to three weeks. Several transfers were made to the respective medium with antifungals in order to obtain pure colonies. All samples were preliminary classified using morphological characteristics and Gram staining (Brenner et al. 2005).

Details of materials and methods for this section were performed using the same procedure described previously in Chapter 2 (page 12).

3.2.3 DNA extraction, 16S rDNA gene amplification and sequencing

DNA extraction and 16S rDNA gene amplification from the isolated *Uca rapax*'s hindgut microflora was performed. We extracted the DNA from all isolates from pure cultures using the CTAB chemical method. PCR amplification of 16S rDNA gene reaction for a final volume of 25 µL was carried out using the DNA template, 0.8x PCR buffer, 3 nM $MgCl_2$, 0.6 mM of each primer, 0.16 mM dNTPs and 5U Taq polymerase per reaction. We used the universal bacterial primers 27F and 1492R (Lane, 1991) and the following thermal parameters: 95°C: 3 min, 30x (95°C: 45 sec), 52°C: 45 sec, 72°C: 1.5 sec and 72°C: 10 min for the amplification. All PCR products were sequenced at the High-Throughput Genomics Unit of the University of Washington, Seattle, WA.

Details of materials and methods for this section were performed using the same procedure described previously in Chapter 2 (page 13).

3.2.4 Diversity of hindgut microflora: Data Analysis

3.2.4.1 Diversity Indices

Microbial community diversity from the hindgut was assessed with diversity indices. We used the Simpson index (D) as an index of diversity, and the Shannon-Wiener index (H') as an index of evenness. To calculate the biodiversity indices of Simpson and Shanon-Wiener we used the free software application EstimateS 8® (Colwell, 2009).

Details of materials and methods for this section were performed using the same procedure described previously in Chapter 2 (page: 14).

3.2.4.2 Bacterial accumulation curve and comparison of microbial communities

The bacterial accumulation curve was performed to calculate the expected richness of species by the number of samples or total individuals. We used the free software application EstimateS 8® (Colwell, 2009) to obtain the expected species accumulation (Mao Tau values) and number of individuals collected, and then plotted the accumulation curve using Excel® (Microsoft® Excel®, 2010) program.

The microbial communities were compared between seasons (wet and dry) and between environments (hindgut vs. mangrove detritus) to determine if there were significant differences. We used the phylogenetic method implemented in UniFrac® (Lozupone et al. 2005) to compare the microbial communities, specifically the UniFrac® Significance analysis with 100 permutations.

Details of materials and methods for this section were performed using the same procedure described previously in Chapter 2 (page 15).

3.2.4.3 Sequence Analysis

Sequences of the 16S rDNA gene (amplified with universal primers 27F and 1492R) were edited and analyzed using Sequencer 4.8® (Gene Codes, Ann Arbor, MI). Then, GenBank® (Benson et al. 2013) searches with BLASTn® (Altschul et al. 1990) were performed to identify the closest available sequences. Phylogenetic analysis and automatic sequence alignment was conducted in MEGA5® (Molecular Evolutionary Genetics Analysis) (Tamura et al. 2011). Phylogenetic trees were inferred by Neighbor-Joining and branch support by bootstrap of 5,000 pseudoreplications.

Details of materials and methods for this section were performed using the same procedure described above in Chapter 2 (page 15).

3.2.5: Scanning electron microscopy (SEM)

Six male crabs were collected for SEM preparation. The crabs were allowed to depurate their gut contents for 3 hours in 0.2 µm filtered mangrove water in individual containers (Gulmann, 2004). The hindgut was dissected and fixed in 1.5 mL of 4% glutaraldehyde in 0.1M phosphate buffer (pH 7.2). Then dehydrated in a series of ethanol dilutions (10-100% solutions) prior the critical point dryer process. Finally, the hindguts samples were coated with gold palladium and examined through SEM (Jeol 5410LV model) at the Microscopy Center of the Biology Department of the University of Puerto Rico -Mayagüez Campus.

3.3 Results

3.3.1 Bacterial communities associated with the hindgut of *Uca rapax* in the wet and the dry seasons

Using culture-dependent methods we identified and characterized a total of 354 strains from the microflora associated the hindgut of *Uca rapax* in both seasons (including pre-sampling) [Appendices: G-H]. In the wet season, we identified 120 bacteria which represent 22 genera (Table no. 3.1). The bacterial genera were Firmicutes (42%), Actinobacteria (41%), Proteobacteria (16%) and Bacteroidetes (1%). The three most abundant genera were *Bacillus* (29%), *Microbacterium* (17%) and *Streptomyces* (10%). In the dry season, we identified 116 bacteria which represent 16 genera (Table no. 3.1). The bacterial genera were the Firmicutes (60%), Actinobacteria (24%) and Proteobacteria (16%). The three most abundant genera were *Bacillus* (50%), *Streptomyces* (9%) and *Microbulbifer* (7%). To isolate all the possible bacterial diversity from the hindgut, we used four different culture media with different nutritional properties. The culture media of Chitin agar and Mangrove soil presented the greater diversity of genera isolated in the wet season (Figure no. 3.1) and the culture media of Marine agar and Mangrove soil presented the greater diversity of genera isolated in the dry season (Figure no. 3.2). The genus *Bacillus* (peach color) was isolated from all the culture media, being the most abundant (36%) of all the bacterial isolated from both seasons and pre-sampling; these results are congruent with mangrove soil bacteria isolates.

Table no. 3.1: Bacteria genera isolated from the hindgut of *Uca rapax* in the wet and the dry seasons.

Season	Genera of bacteria
Wet	*Bacillus, Microbacterium, Streptomyces, Brevibacillus, Cellulosimicrobium, Pseudomonas, Gordonia, Lysinibacillus, Paenibacillus, Paracoccus, Vibrio, Achromobacter, Cellulomonas, Isoptericola, Klebsiella, Rhodococcus, Bosea, Exiguobacterium, Echinicola, Enterobacter, Geobacillus* and *Serratia*
Dry	*Bacillus, Streptomyces, Microbulbifer, Isoptericola, Paenibacillus, Vibrio, Halobacillus, Gordonia, Microbacterium, Lysinibacillus, Marinobacter, Rhodococcus, Ruegeria, Brachybacterium, Halomonas* and *Micrococcus.*

In total we isolated 39 bacterial genera (from 354 strains) from nine samples from the hindgut of *Uca rapax* (Figure no. 3.3). Nine hindgut bacterial genera were present in both seasons: *Bacillus, Microbacterium, Streptomyces, Isoptericola, Vibrio, Gordonia, Lysinibacillus, Rhodococcus* and *Paenibacillus,* while 30 genera were isolated in one of the seasons. Pre-sampling corresponds to the end of the wet season (November), adding six bacteria genera (*Exiguobacterum, Brevibacillus, Enterobacter, Cellulosimicrobium, Pseudomonas* and *Cellulomonas*) that were isolated also from the wet season (Figure no. 3.3).

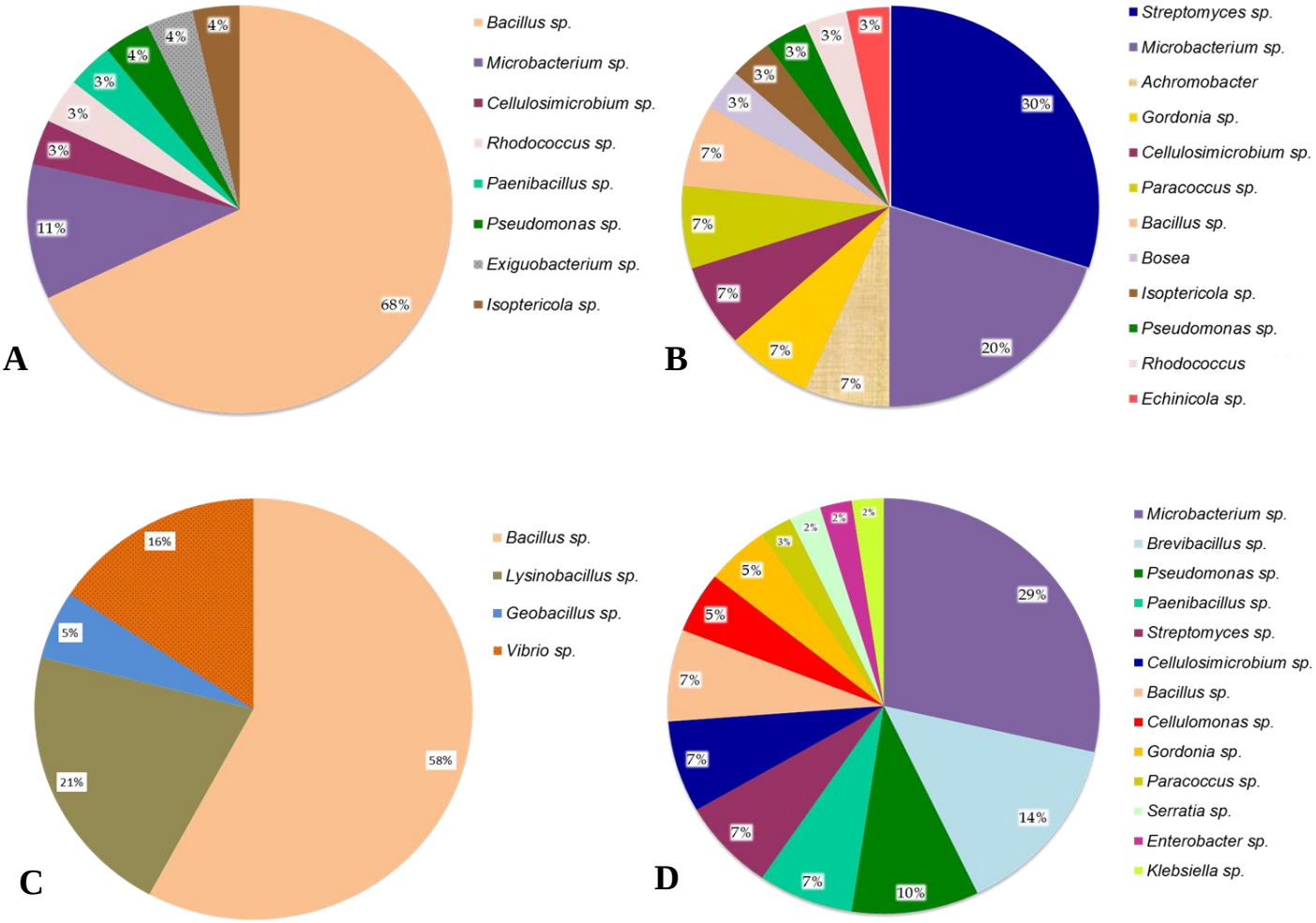


Figure no. 3.1: Genera of bacteria isolated from the hindgut of *Uca rapax* in the wet season. (A) Marine agar, (B) Chitin agar, (C) LB agar, and (D) Mangrove soil agar.

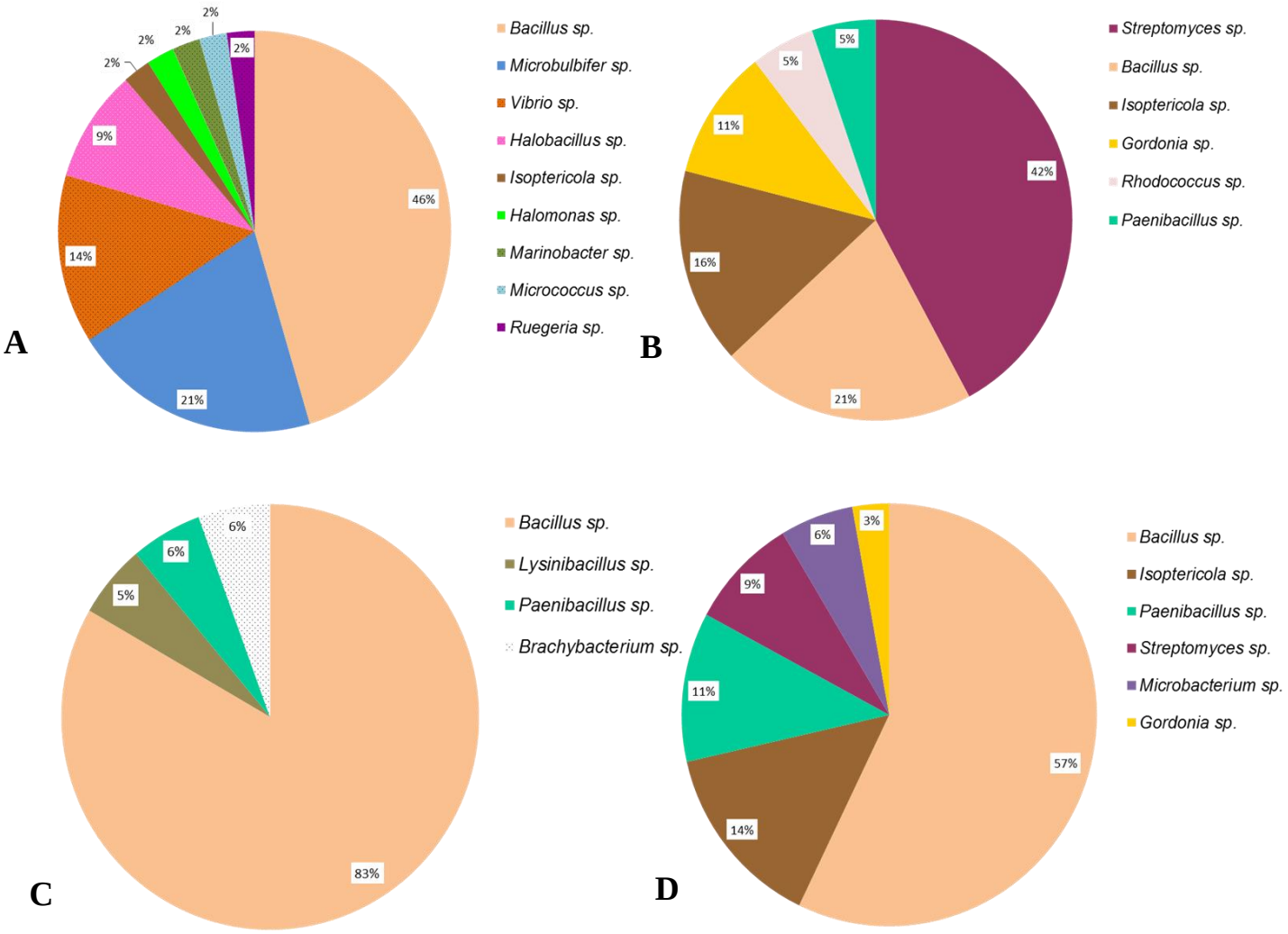


Figure no. 3.2: Genera of bacteria isolated from the hindgut of *Uca rapax* in the dry season. (A) Marine agar, (B) Chitin agar, (C) LB agar, and (D) Mangrove soil agar.

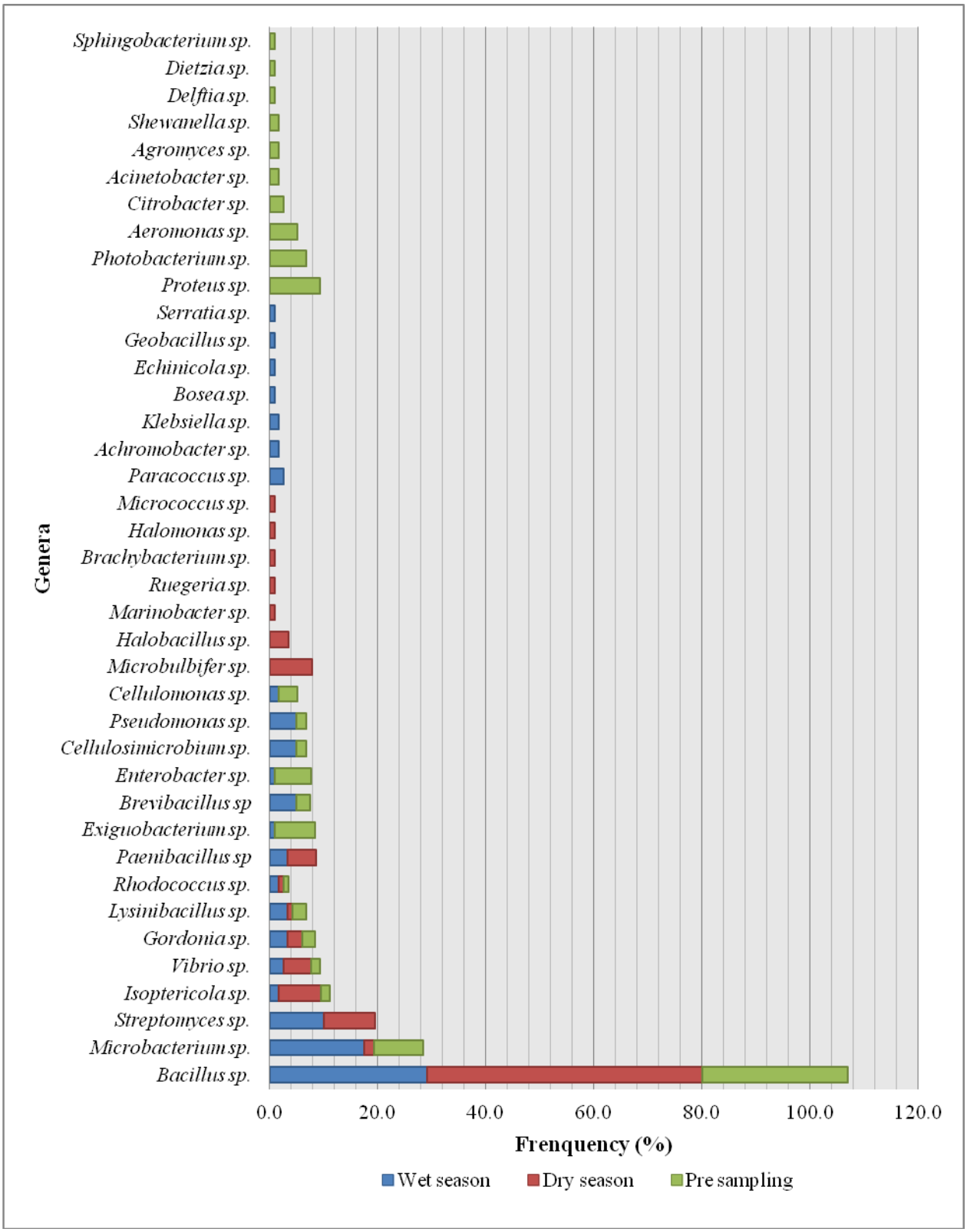


Figure no. 3.3: Microflora genera abundance isolated from the hindgut of *Uca rapax* in the wet and the dry seasons.

3.3.2 Diversity indices and bacterial accumulation curve of the hindgut of *Uca rapax* microflora.

To describe the biodiversity of the cultivable hindgut microflora we calculated diversity indices (Simpson's and Shannon-Weiner's indices) and also created a bacterial accumulation curve of the 354 strains isolated from all sampling. Simpson's index of dominance (D) measures the probability that two individuals randomly selected from a sample will belong to the same species (genera); or to different species (Simpson's index of diversity [S= D-1]). Diversity index value ranges is 0 and 1; in which 0 represents low diversity and 1 represent high diversity (Simpson, 1949). The entire hindgut microflora (39 genera from 354 strains) presented a Simpson's index of diversity value of 0.8484 indicating high diversity in the community (Table no. 3.2). We also calculated Shannon-Weiner's index (H'); according to Brown et al. (2001) this index measures the average degree of uncertainty (synonymous with diversity) of predicting the species of a given individual picked at random from a community; the index varies from a value of 0 for communities with only a single species to high values for communities having many species. The Shannon's index value was 2.4578 indicating that the community is highly diverse (Table no. 3.2).

In order to determine the sampling effort realized we created an accumulation curve (Figure no. 3.4). The results suggest more sampling effort is needed because the curve does not reach an asymptote.

Table no. 3.2: Diversity indices of the microflora from the hindgut of *Uca rapax.*

Diversity Indices		
Whole hindgut microflora	-Simpson's Index of Dominance (D)	0.1516
	-Simpson's Index of Diversity (S=1-D)	**0.8484**
	-Simpson's Reciprocal Index (1/D)	6.5963
	-Shannon-Weiner's Index (H')	**2.4578**
Wet hindgut microflora	- Simpson's Index of Diversity (S=1-D)	0.8637
	-Shannon-Weiner's Index (H')	2.3000
Dry hindgut microflora	- Simpson's Index of Diversity (S=1-D)	0.8340
	-Shannon-Weiner's Index (H')	2.4200

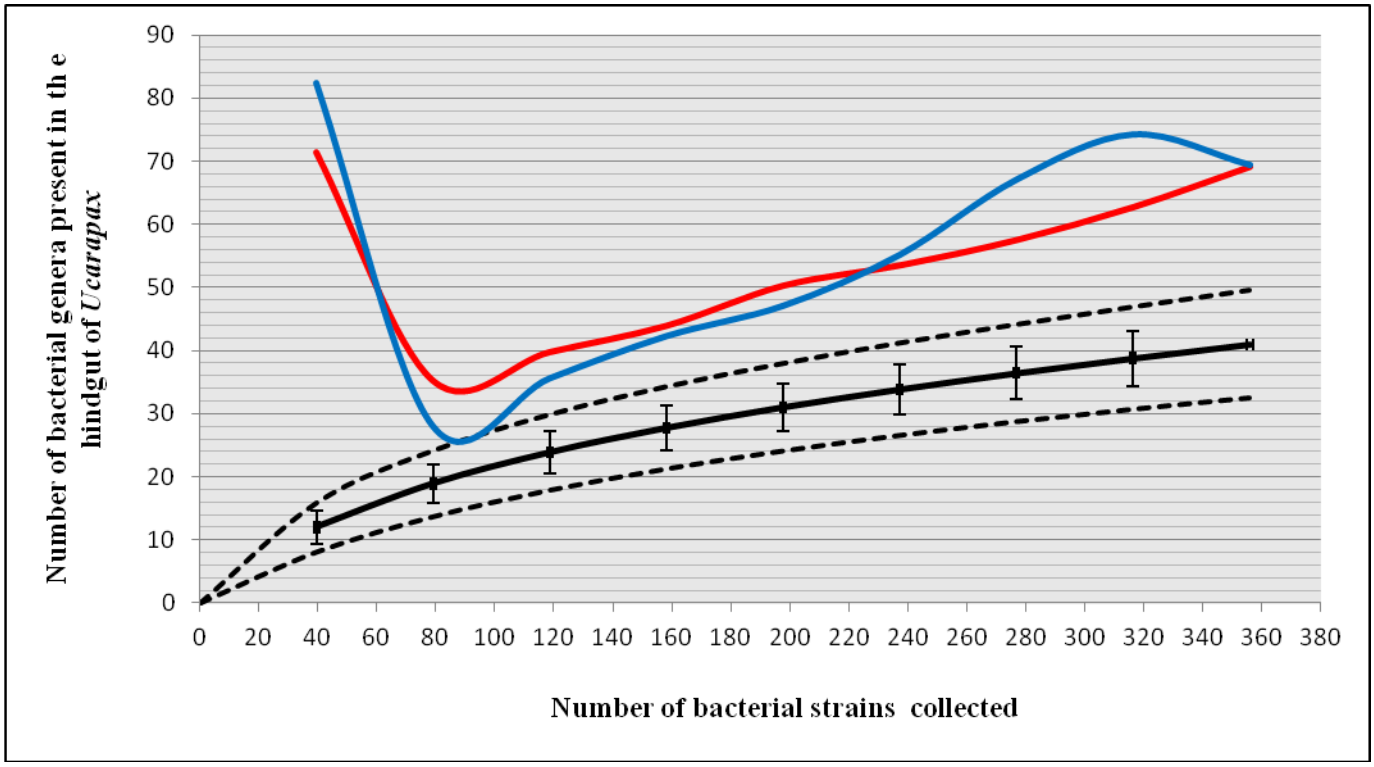


Figure no. 3.4: Sample-based bacterial accumulation curve displaying number of bacterial strains collected against number of identified bacterial genera (Mao Tau [Sobs] values) present in the hindgut of *Uca rapax*. Dashes lines represent a 95% of confidence intervals. And the corresponding richness estimators: Chao 2 Mean (red line) and ICE (incidence-based coverage estimator) Mean (blue line).

3.3.3 Comparison between hindgut microfloras associated in the wet and the dry seasons

Hindgut microfloras (39 genera from 354 strains) were compared in Unifrac® between the wet season (HG_W) and the dry season (HG_D). The results indicated that there are significant differences between seasons (Table no. 3.3).

Table no. 3.3: Matrix showing p-values from comparisons of *Uca rapax*'s hindgut microflora in the wet and the dry seasons using Bonferroni corrected data. Colors indicate the significant difference on a scale defined by Unifrac®.

	HG_D	HG_W
HG_D		<=0.01
HG_W		

Color	Description
	(< 0.001) Highly significant
	(0.001-0.01) Significant
	(0.01-0.05) Marginally significant
	(0.05-0.1) Suggestive
	(> 0.1) Not significant

When comparing the microbial communities from both environments (mangrove soil and from the hindgut of *Uca rapax*) and from both seasons, five out of six pairwise comparisons of p-values (uncorrected data) indicated that there are highly significant differences (Table no. 3.4). When comparing hindgut microflora against mangrove soil bacteria from the dry season, the results indicated that there are no significant differences.

Table no. 3.4: Matrix showing p-values of multiple comparisons of *Uca rapax*'s hindgut microflora and mangrove soil microflora of the wet and the dry seasons using uncorrected data. Colors indicate the significant or no significant differences between each pair on a scale defined by Unifrac®.

	HG_D	HG_W	MD_D	MD_W
HG_D		0.0000	0.2600	0.0000
HG_W			0.0000	0.0000
MD_D				0.0000
MD_W				

Color	Description
	(< 0.001) Highly significant
	(0.001-0.01) Significant
	(0.01-0.05) Marginally significant
	(0.05-0.1) Suggestive
	(> 0.1) Not significant

3.3.4 Phylogenetic analysis of the hindgut of *Uca rapax* microflora

We created a 16S rDNA phylogenetic tree comparing the entire hindgut microflora from both seasons combined (Figure no. 3.5). The principal objective was to determine the phylotypes represented in the hindgut, to compare wet and the dry season's taxa, and to detect season-specific clades. Our results indicated that there were distinctive clades formed only by sequences from the same season, which means that taxa composition differs between seasons. The phylotypes isolated in this study were Actinobacteria, Proteobacteria, and Firmicutes (clockwise).

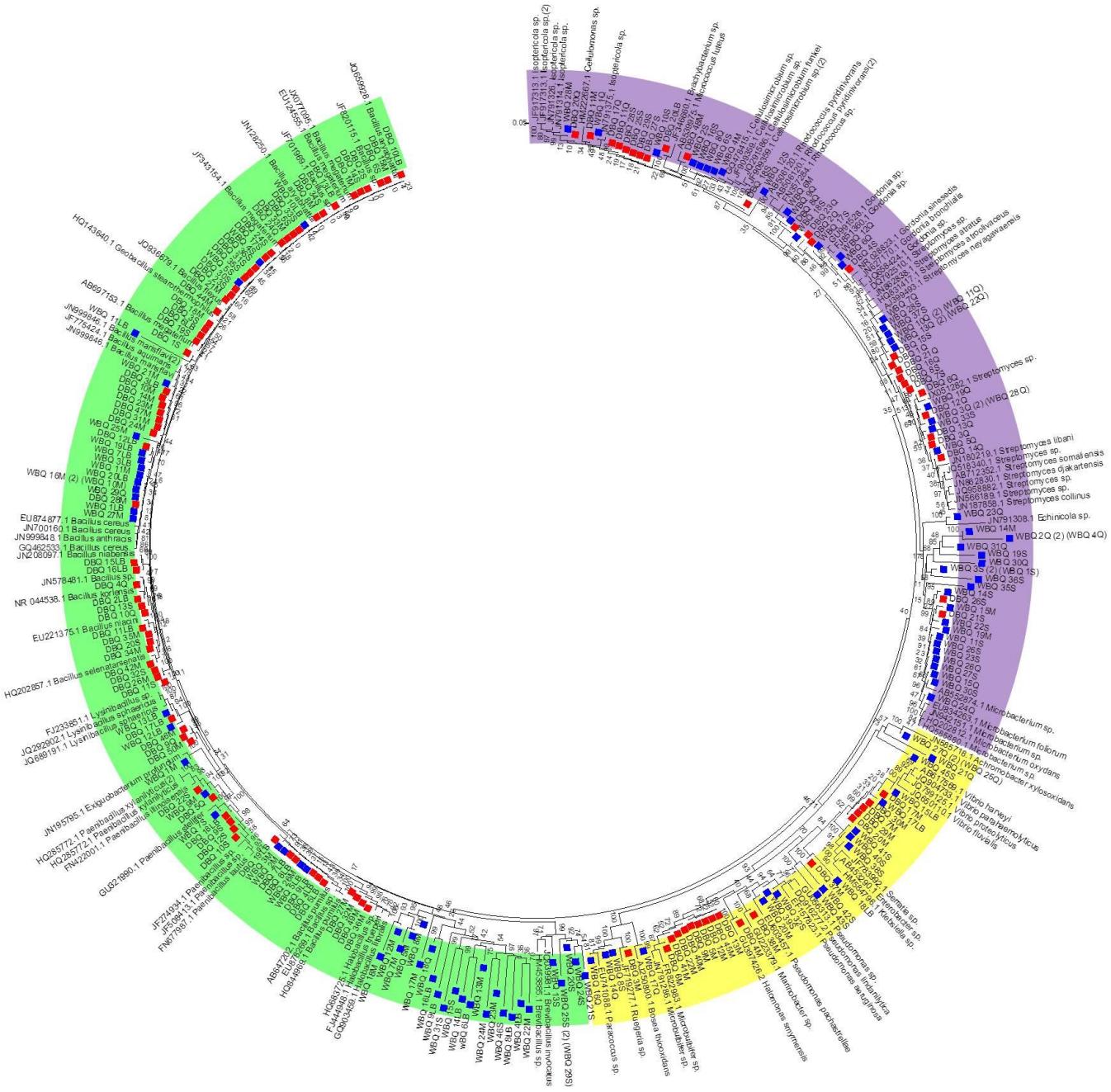


Figure no. 3.5: Phylogenetic Neighbor-joining tree based of 16S rDNA gene sequences isolated from *Uca rapax*'s hindgut (sequences from the wet season are blue colored and sequences from the dry season are red colored) and closely related species found in GenBank®. The phylotypes isolated in this study were Actinobacteria (purple shade), Proteobacteria (yellow shade) and Firmicutes (green shade). The phylogeny is based on partial 16S rDNA sequences of approximately 1,000bp. The numbers at the nodes indicate bootstrap support values (>50%) based on 5,000 pseudoreplicates. The scale bar corresponds to 0.05 substitutions per site.

3.3.5 Specific microflora associated with *Uca rapax*

We identified the genera of bacteria that were consistently isolated from the same or both environments (hindgut or mangrove soil) and by season (Figure no. 3.6). *Bacillus*, *Microbacterium*, *Isoptericola*, *Gordonia*, *Lysinibacillus* and *Rhodococcus* were consistently isolated from the hindgut of *Uca rapax* and mangrove soil in both seasons. *Mycobacterium*, *Nocardia* and *Novosphingobium* were only isolated from mangrove soil in both seasons. *Microbulbifer* and *Brevibacillus* were isolated from mangrove soil (in both seasons), but were also reported in the hindgut microflora in the dry season and the wet season, respectively (Figure no. 3.3-3.6). *Streptomyces* and *Paenibacillus* were isolated from *Uca rapax*'s hindgut (in both seasons), but were also found in the dry season from mangrove soil (Figure no. 3.3-3.6).

In order to identify specific microflora associated with the hindgut of *Uca rapax*, we compared the bacterial genera present in the hindgut against the ones in mangrove soil from the same season. Only *Vibrio* was unique to the crab hindgut, isolated in both seasons, but never found in the mangrove soil.

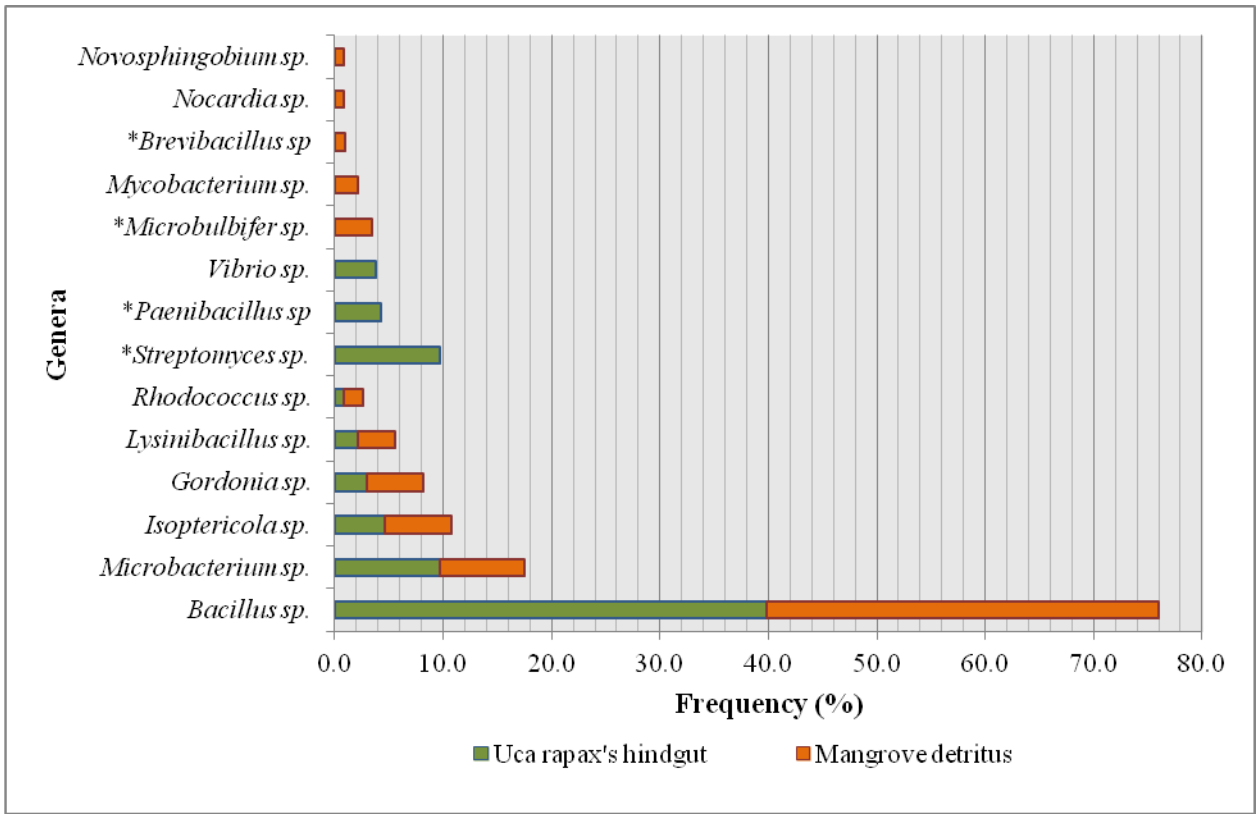


Figure no. 3.6: Bacterial genera abundance isolated from mangrove soil and *Uca rapax*'s hindgut in both seasons. *Brevibacillus* and *Microbulbifer* were also reported in the hindgut but not in both seasons. *Streptomyces* and *Paenibacillus* were also reported in the mangrove soil but not in both seasons.

3.3.6 Scanning electron microscopy analysis of the microflora associated with the hindgut of *Uca rapax*

Different bacterial morphologies were found in the hindgut associated with the gut lining. Filamentous-shaped bacteria were associated with the anterior region (Figure no. 3.7 [G-I]). Detritus, plant material and dense groups of cocci-shaped bacteria were found on the internal hindgut wall (Figure no. 3.7 [A-C]). Also, rod-shaped bacteria and other non-identified bacteria morphologies were found attached to the posterior region of the hindgut (Figure no. 3.7 [D-F]). We observed other non-identified morphologies in the posterior region of the hindgut, which might be detritus, plant material and other associated microorganisms (Figure no. 3.8).

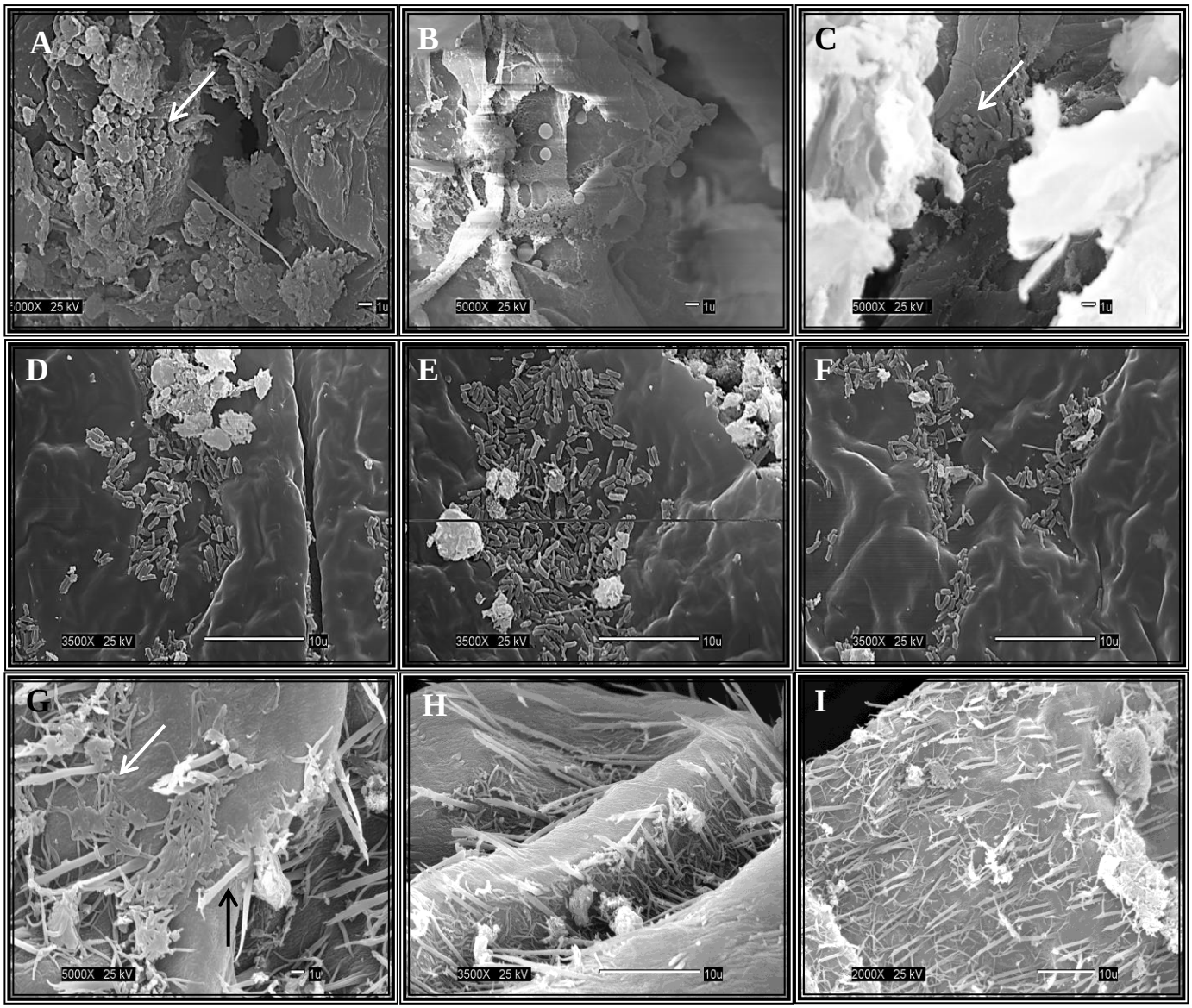


Figure no. 3.7: Scanning electron microscopy photographs of the hindgut of *Uca rapax*. (A-C): Detritus or plant material and cocci-shaped bacteria (white arrow) localized on internal hindgut's wall. (D-F): Posterior region of hindgut lining with attached rod-shaped bacteria. (G-I): Anterior region of chitin-lined hindgut (black arrow) with filamentous-shaped bacteria (white arrow).

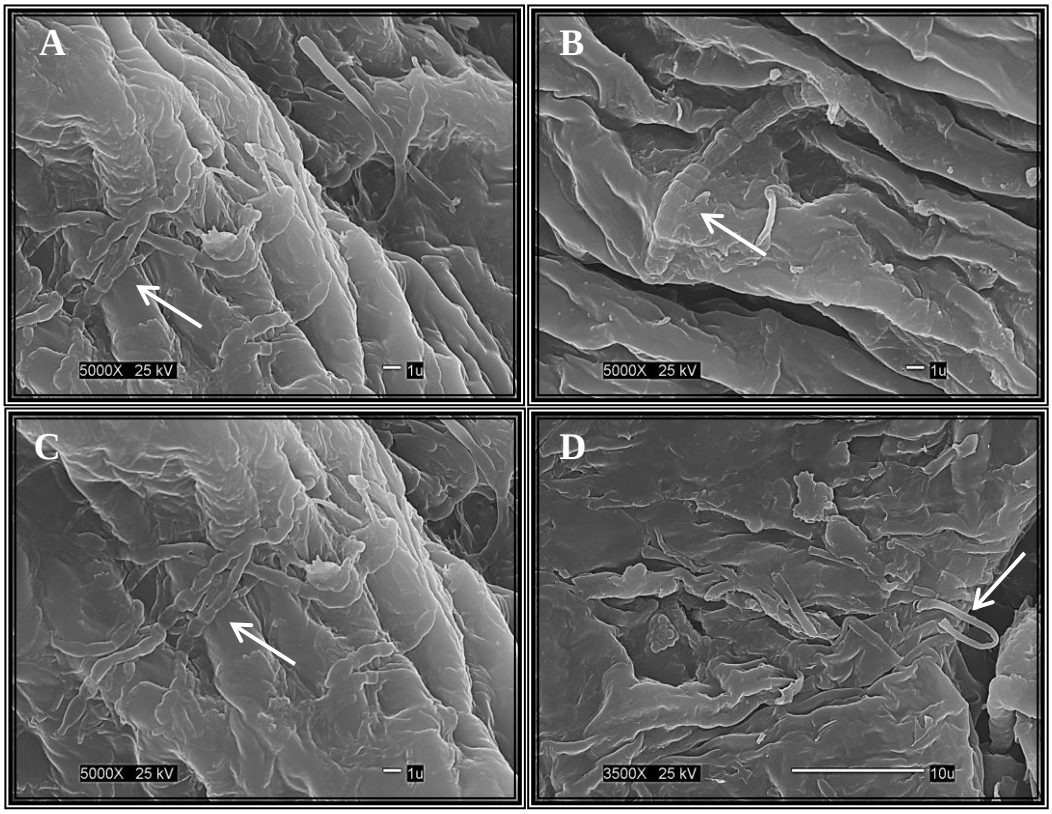


Figure no. 3.8: Scanning electron microscopy photographs of hindgut posterior region of *Uca rapax*. (A-D): Might be detritus, plant material and/or other microorganisms.

3.4 Discussion and Conclusion

Fiddler crabs ecological role is to recycle organic matter by processing detritus, also by burrowing deep into the mud they create a maze of tunnels that aerate the estuarine soil and promote aerobic conditions. They are deposit-feeders, detritus forms part of their diet, though sand filtered may contain particles algae, archaea, ciliates, fungi and bacteria. Some authors claim that gut microbes may contribute to the host nutritional fitness, especially by increasing the extracellular enzymes to degrade organic matter. We studied the bacteria, especially from the

hindgut of marine invertebrates, because they are not competing directly with their host for the uptake of digested compounds (Lau et al., 2002). Thus, it might represent a mutualistic nutritional relationship.

In this study, we observed a similar pattern of diversity and abundance of bacteria isolated from the hindgut (from both seasons) and from the mangrove soil. As before, the culture media of Chitin agar and Mangrove soil agar presented a greater richness of isolated genera, possibly because of the nutritional properties similar to those in the digestive system of *Uca rapax* (Chitin agar) and mangrove sediments (Mangrove soil agar) from which they filter food. Although similar at first glance, we found significant differences between the communities of mangrove bacteria and the hindgut microflora in five out of six pairwise comparisons in Unifrac®. Bacterial communities associated with the hindgut of *Uca rapax* were different from the ones in the soil only in the wet season. Dominant phytotypes were Actinobacteria and Firmicutes for both environments, which suggest that the differences between them are due to the diversity of genera. These bacteria possibly form endospores to survive and; when placed in culture media, they will outcompete and grow faster than other bacteria (gram negative) that do not have these stress adaptations. This effect could explain why the Firmicutes were so abundant in this study. However, when only comparing the communities of hindgut microflora against those from the mangrove bacteria from the dry season, the results show no significant differences between communities possibly because of water scarcity, which prevent even distribution and survival of other bacterial groups. The biodiversity indices of Simpson (0.8484) and Shannon-Weiner (2.4578) indicate a high diversity in the hindgut microflora based on culture dependent methods. Nonetheless, the bacterial accumulation curve suggests more sampling effort is needed to obtain the real richness that can found in the hindgut of *Uca rapax* when we only consider

cultivable bacteria. According to the richness estimator, Chao 2, the accumulation curve represents a 59% of the 0.1% of cultivable diversity found in the hindgut. These results are due to the limitation of culture media, which represent the hindgut environment.

The hindgut of the fiddler crab, *Uca rapax* was investigated using scanning electron microscopy (SEM). Within the lumen of the hindgut: rods, filamentous and cocci-shaped bacteria were observed. The microbial populations were dominated by dense groups of cocci-shaped bacteria. Based on those results and the literature, we hypothesize that the filamentous and cocci-shaped bacteria found were from pleomorphic *Bacillus* species, which under nutrient scarcity and competition, size reduction and lower metabolic rate might have supported their survival (Justince et. al. 2008). Another explanation is that these bacteria really are cocci, and that *Bacillus* abundance is limited to the presence of detritus in the hindgut. We also observed other non-identified morphologies that were only found in the posterior region of the hindgut, which might be detritus, plant material or other microorganisms such as fungi or ciliates. Our results agree with those reported in *Uca pugnax* by Gulmann (2004), although the filamentous bacteria from the posterior hindgut are described as curved rod-shaped bacteria, a description that does not match our observations.

To identify specific bacteria communities associated with the hindgut of *Uca rapax*, we compared the bacteria genera present in both mangrove and hindgut environments. *Vibrio* was the only exclusive isolate from the hindgut (from both seasons and pre-sampling) never recovered from mangrove soil. Despite using Thiosulfate-Citrate-BileSalts-Sucrose (TCBS) medium, selective for *Vibrio* species (Farmer et al. 2006), *Vibrio* was not isolated from mangrove soil. *Vibrio* was present in the hindgut of *Uca rapax* at a low frequency (3.8%), but it is unlikely to represent a symbiotic strain. *Vibrio* species have been commonly described in

bioluminescent symbiosis with marine animals, such as fishes and squids. The stable association between the bobtail squid (*Euprymna scolopes*) and *Vibrio fischeri* is a horizontally transmitted symbiosis (acquires the symbiont from the surrounding environment), where the squid cultures dense populations of luminous bacteria in their light-organ to avoid predators during their nocturnal behavior (Nyholm et al. 2004; Urbanczyk et al. 2010). Using culture-dependent methods we were not able to detect potential symbionts associated with *Uca rapax*'s hindgut. The characterization of symbiont microflora must be analyzed and described *in vivo*, e.g. GFP-encoding plasmid (Nyholm et al. 2000); because the host must provide mechanisms for the enrichment and harvesting of specific microorganisms (symbionts), which are scarce in the environmental microbiota (Nyholm et al. 2004). If there are symbiotic *Vibrio* species associated with the hindgut of *Uca rapax*; and we provide a hypothetical culture media with the nutritional characteristics of the hindgut environment, we could expect to isolate a dense abundance of *Vibrio* mediated by quorum sensing and not at low frequency as described in our results.

Bacterial populations associated with the hindgut of *Uca rapax*, whether they are transients or residents (symbionts), represent a mutualistic nutritional relationship in which they provide benefits for the host's fitness. The mangrove soil and hindgut bacteria play important roles in recycling nutrients, hence benefiting the stability and conservation of mangrove ecosystems.

Chapter 4: Cellulose degradation of hindgut microflora

4.1 Introduction

Mangrove soils are mainly composed of lignocellulose components such as leaves and wood, which are degraded by microorganisms to produce detritus (Alongi et al. 1989). According to Holguin et al. (2001) detritus is defined as organic matter in the active process of decomposition. Odum et al. (1975) investigated the microscopic communities of decomposing mangrove leaves and revealed a complex community composed of fungi, bacteria, protozoa, archaea and microalgae.

The cellulose (β-1,4-glucan) is one of the most abundant carbohydrates and the primary product of photosynthesis (Thatoi et al. 2013) in mangrove soils, which means that cellulase activity (endo-β-1,4-glucanase, exo-β-1,4-glucanase and β-glucosidase) is important to the nutritional fitness of detritus feeders such as fiddler crabs. Cellulases are secreted by microorganisms, yet it is not known if fiddler crabs are able to do so as well, although cellulose degradation is significant because they need the carbon source. According to Dall et al. (1983), crustaceans must profit of this carbon source, but it is not clear if the cellulase activity by microorganisms occurs in the gut (by the presence of symbionts) or in the food (soil) (by the presence of transient microflora) prior to ingestion.

In this chapter, we studied cellulose degradation (under laboratory conditions) in an attempt to understand the capacity of the hindgut microflora of *Uca rapax* to degrade mangrove plant material or detritus. We used cellulolytic enzyme assays using carboxymethylcellulose as the only carbon source to identify cellulase producing bacteria.

4.2 Materials and Methods

4.2.1 Cellulolytic enzyme assays

Carboxymethylcellulose (CMC) assay serves as a good indicator of cellulolytic ability of microorganisms. Carboxymethylcellulose is a substrate for the detection of endo-β-1,4-glucanase activity and β-glucosidase activity (Pointing, 1999).

4.2.1.1 Qualitative screening

Bacteria were inoculated in triplicate and incubated at 25°C for 72 hours on CMC agar (0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.2% carboxymethylcellulose (CMC) sodium salt, 0.02% peptone, and 1.7% agar). Then, plates were flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 minutes (Kasana et al. 2008) to better observe the degradation zone (clearing) around the colony. All positive isolates with degradation zone were evaluated semi-quantitatively.

4.2.1.2 Semi quantitative screening

After the qualitative screening, we conducted a semi-quantitative analysis of all cellulase producers to determine cellulose degradation capacity of these isolates. Bacteria were inoculated and incubated at 25-30°C for 48-72 hours on Lenox (LB) nutrient broth or Marine nutrient broth at 200 rpm. Then, through cell density absorbance we standardized the inoculum using the following equation:

$$inoculum\ mL = \frac{DESIRED\ OD}{REAL\ OD}\ X\ nutrient\ broth\ (mL)$$

The desired-OD represents an absorbance value of 0.075 (*E. coli* has an absorbance value of 0.025 after 24 hours) while the real-OD represents the absorbance value of the isolate at a wavelength of 600nm. This equation allows us to determine the quantity of milliliters of inoculum required for a dilution of 5mL of nutrient broth, so that all colonies have approximately the same number of cells. Later, sterile filter paper disks (7 mm diameter) soaked in the bacteria suspension were incubated in 25 mL CMC agar at 25-30°C for 72 hours. This was done in triplicate. We measured the zone of degradation (mm) of each triplicate after flooding with Gram's iodine.

4.2.2 Non parametric analysis

Shapiro-Wilks test determines the normality of the data; when the p-value is smaller than alpha (0.05), the data do not follow a normal distribution (Shapiro et al. 1965). The Krustal-Wallis test is a non-parametric method for testing whether samples originate from the same distribution, when the p-value is smaller than alpha (0.05) there are significant differences (Krustal et al. 1952). Variance analyses (non-parametric ANOVA by Krustal-Wallis) and multiple comparisons (Tukey test) were conducted with InfoStat® (InfoStat®, 2004) program to determine differences of cellulose degradation capacity between bacterial strains.

4.3 Results

4.3.1 Qualitative analysis

We compared the microbial communities from the hindgut microflora and the mangrove soil in each season, to determine which of those bacteria genera were only found in the hindgut of *Uca rapax*. In the wet season, we identified 38 strains, which represent 13 genera (*Streptomyces*, *Pseudomonas*, *Paenibacillus*, *Vibrio*, *Paracoccus*, *Achromobacter*, *Klebsiella*, *Exiguobacterium*, *Bosea*, *Geobacillus*, *Echinicola*, *Enterobacter* and *Serratia*). Following the qualitative assay, 82% of strains studied were positive for cellulose degradation (Figure no. 4.1). In the dry season samples, we identified 12 strains representing six genera (*Vibrio*, *Microbacterium*, *Halomonas*, *Micrococcus*, *Brachybacterium* and *Ruegeria*), where 83% of them were positive cellulase producers (Figure no. 4.1). The principal phylotypes represented in both season were Proteobacteria (>50%) and Actinobacteria (>30%).

In the wet season, the positive cellulase producers were *Streptomyces* (Figure no. 4.2 [A]), *Pseudomonas*, *Paenibacillus*, *Vibrio*, *Paracoccus* (Figure no. 4.2 [B]), *Achromobacter*, *Klebsiella*, *Bosea*, *Geobacillus*, *Echinicola* and *Enterobacter*. In the dry season, the positive cellulase producers were *Vibrio* (Figure no. 4.2 [C]), *Microbacterium*, *Halomonas*, *Micrococcus*, *Brachybacterium*.

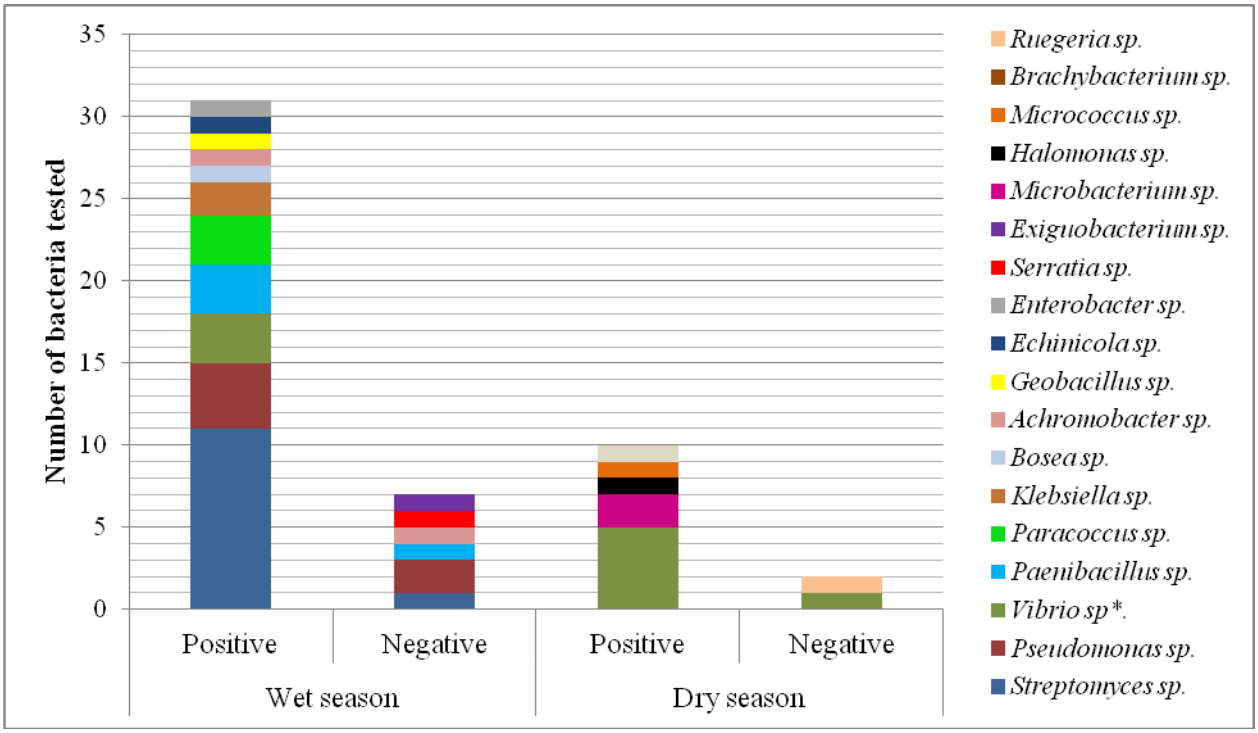


Figure no. 4.1: Qualitative screening to determine the presence of cellulase producing bacteria from the hindgut of *Uca rapax*. The analysed bacteria were the genera found only in the hindgut (absent in the mangrove soil).

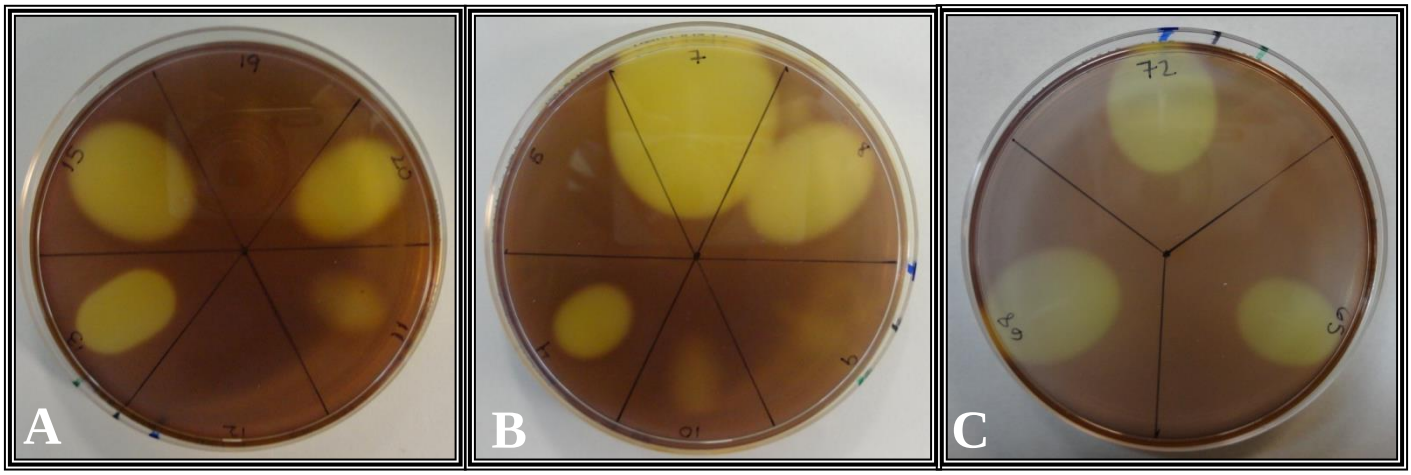


Figure no. 4.2: Cellulase producing bacteria (degradation halo) from the hindgut of *Uca rapax* inoculated in CMC agar and flooded in GRAM's iodine. (A): *Streptomyces* strains from the wet season. (B): *Paracocccus* strains (#4, #7 and #10) from the wet season. (C): *Vibrio* strains from the dry season.

4.3.2 Semi quantitative analysis

We conducted a semi-quantitative analysis of all positive cellulase producers to determine cellulose degradation capacity of the isolates. In the wet season, we analyzed 31 strains, in which the genera with greater degradation capacity (>30mm) were *Paracoccus* (strains: 14Q and 8S), *Streptomyces* (strains: 28S, 22Q, 28Q and 19Q), *Geobacillus* (strain 9LB), *Paenibacillus* (strains: 15S and 9M), *Achromobacter* (strain 27Q), *Klebsiella* (strains: 40S and 41S) and *Pseudomonas* (strains: 32S, 39S and 20M) (Figure no. 4.3). In the dry season, we analysed 10 strains, in which the genera with greater degradation capacity (>30mm) were *Brachybacterium* (strain 20LB), *Vibrio* (strain 37M) and *Microbacterium* (strain 21S) (Figure no. 4.4). Figure no. 4.5 shows the differences in degradation halo of different strains from both seasons. *Vibrio* sp. (strain 37M) always showed a lower cellulose degradation capacity when compared to other bacteria genera.

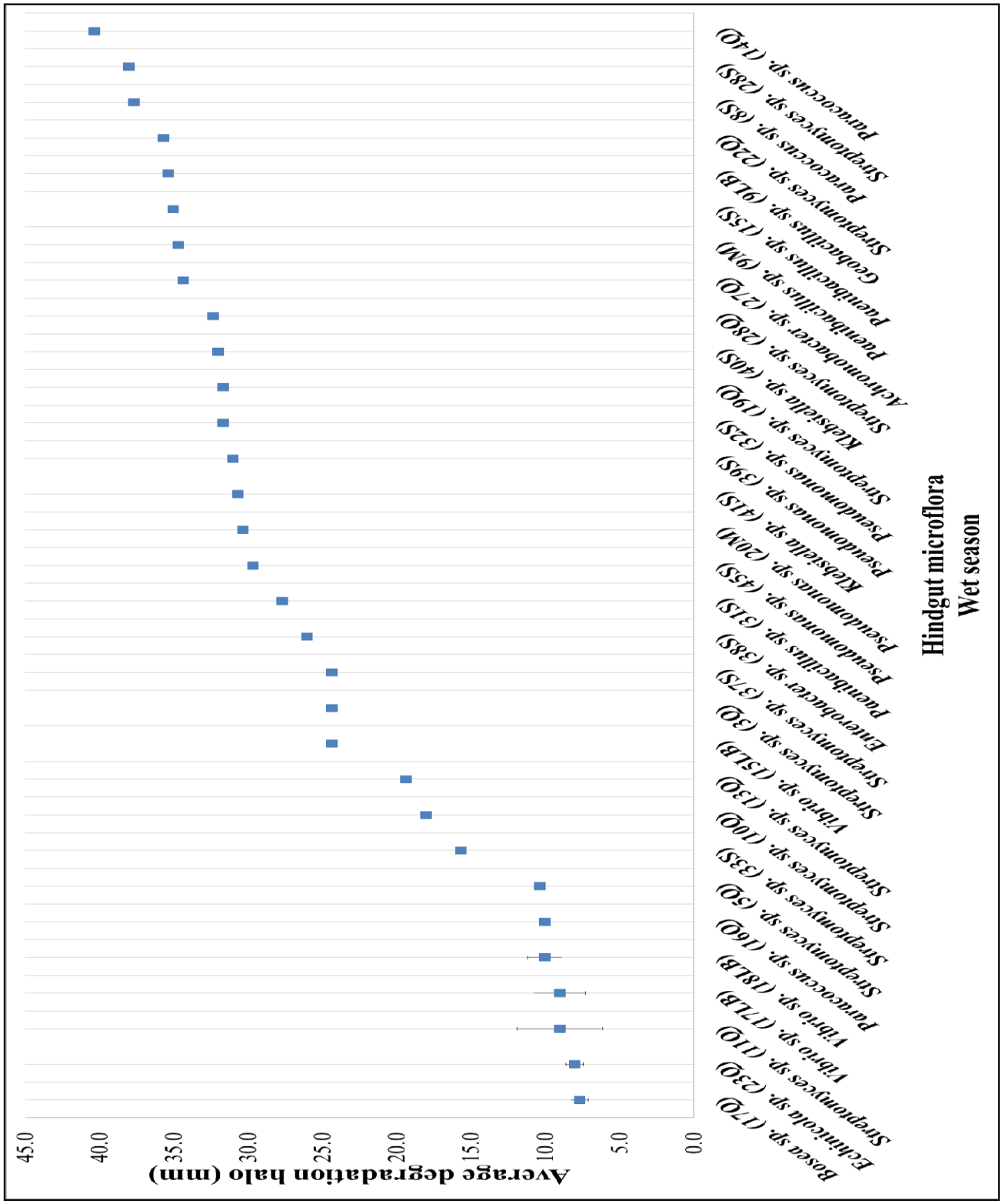


Figure no. 4.3: Average degradation halo (mm) of cellulase producing bacteria from the hindgut of *Uca rapax* in the wet season. Error bars represent the standard deviation.

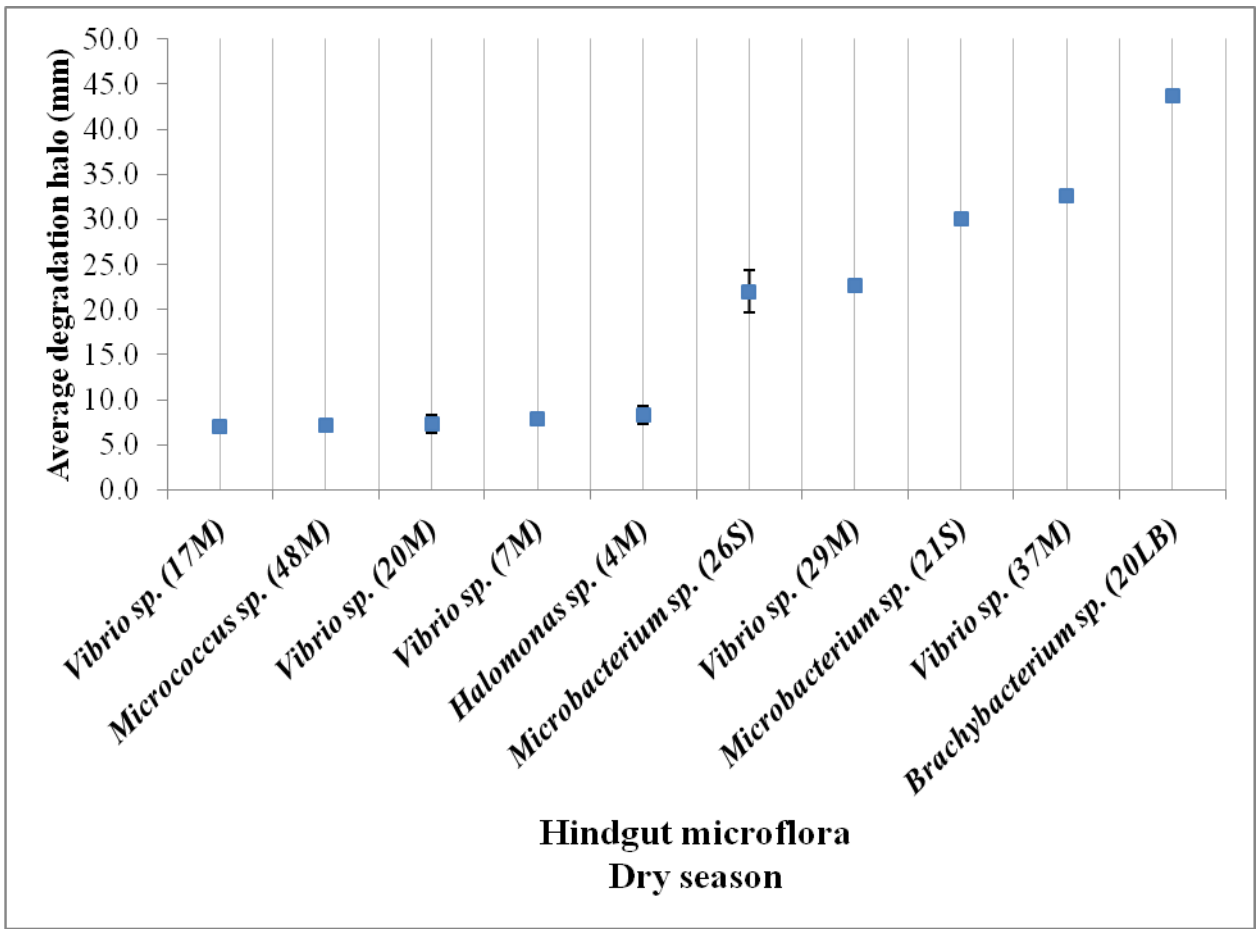


Figure no. 4.4: Average degradation halo (mm) of cellulase producing bacteria from the hindgut of *Uca rapax* in the dry season. Error bars represent the standard deviation.

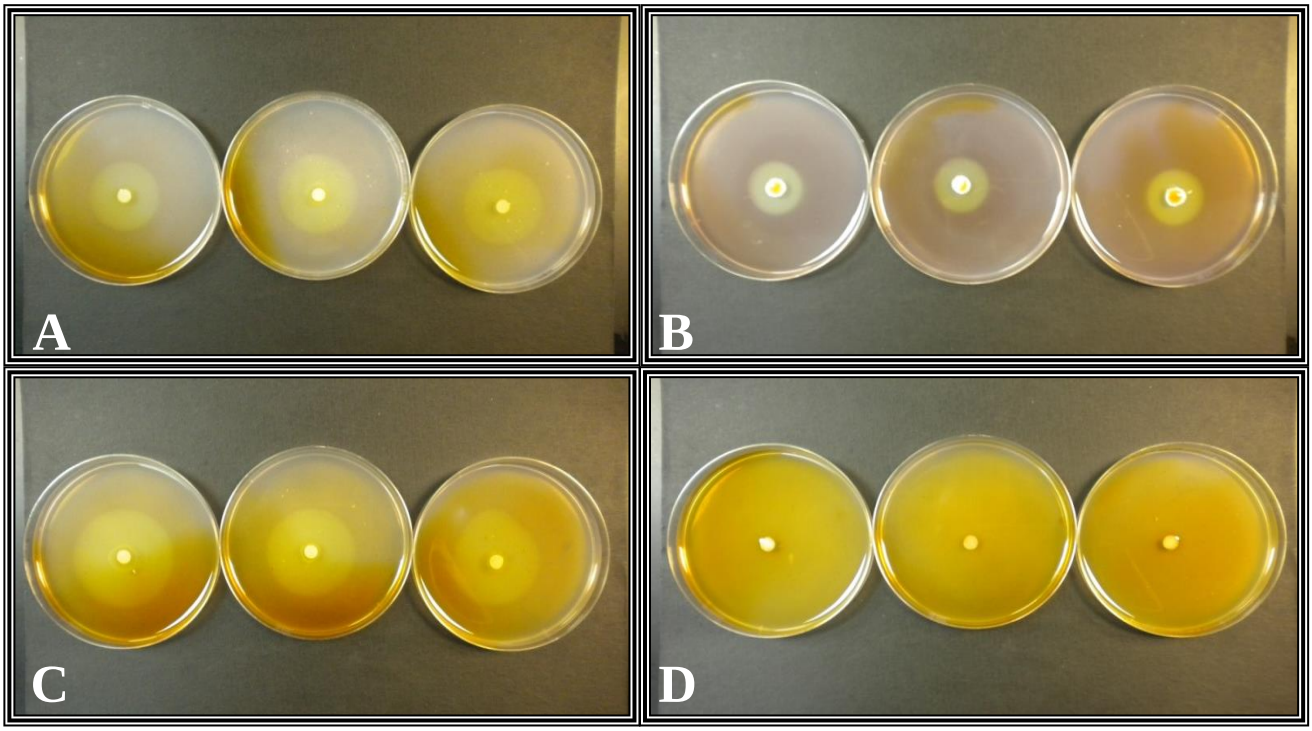


Figure no. 4.5: Semi quantitative screening of cellulase producing bacteria from the hindgut of *Uca rapax*. (A-B): *Achromobacter* (Strain 27Q) and *Streptommyces* (Strain 37S) from the wet season, respectively. (C-D): *Brachybacterium* (Strain 20LB) and *Halomonas* (Strain 4M) from the dry season, respectively.

4.3.3 Statistical analysis

The normality test of Shapiro-Wilks determined that the semi quantitave analysis data were not normally distributed because the p-value was less than 0.05 (<0.0001) (Figure no. 4.6-4.7). Due to the data being not normally distributed we used a non-parametric ANOVA under the Kruskal Wallis test and multiple comparisons (Tukey test) to determine differences of cellulose degradation capacity between bacterial strains. In the wet season, the variance analysis showed a significant difference ($p<0.0001$) and the pairwise comparison using DMS value, all of which showed that there are at least 12 groups with significant differences among them (Figure no. 4.8). In the dry season, the pairwise comparison using DMS value showed that there are at least four groups with significant differences among them (Figure no. 4.9).

Shapiro-Wilks (modified)

Variable	n	Mean	S.D.	W*	p(one tail)
Degradation halo	89	25.04	10.61	0.87	<0.0001

Kruskal Wallis Test

Variable	Strains	N	Means	S.D.	Medians	H	p
Degradation halo	10Q	3	18.00	0.00	18.00	89.52	<0.0001
Degradation halo	11Q	3	9.00	0.00	9.00		
Degradation halo	13Q	3	19.33	0.58	19.00		
Degradation halo	14Q	3	40.33	1.15	41.00		
Degradation halo	15LB	3	24.33	0.58	24.00		
Degradation halo	15S	3	35.00	1.73	36.00		
Degradation halo	16Q	3	10.00	1.00	10.00		
Degradation halo	17LB	3	9.00	0.00	9.00		
Degradation halo	17Q	3	7.67	0.58	8.00		
Degradation halo	18LB	3	10.00	0.00	10.00		
Degradation halo	19Q	3	31.67	1.15	31.00		
Degradation halo	20M	3	30.33	0.58	30.00		
Degradation halo	22Q	3	35.67	0.58	36.00		
Degradation halo	23Q	3	8.00	0.00	8.00		
Degradation halo	27Q	3	34.33	2.08	35.00		
Degradation halo	28Q	3	32.33	0.58	32.00		
Degradation halo	28S	3	38.00	1.73	39.00		
Degradation halo	31S	3	27.67	1.53	28.00		
Degradation halo	32S	3	31.67	0.58	32.00		
Degradation halo	33S	3	15.67	0.58	16.00		
Degradation halo	37S	3	24.33	0.58	24.00		
Degradation halo	38S	3	26.00	1.00	26.00		
Degradation halo	39S	3	31.00	0.00	31.00		
Degradation halo	3Q	3	24.33	0.58	24.00		
Degradation halo	40S	3	32.00	2.00	32.00		
Degradation halo	41S	3	30.67	2.52	31.00		
Degradation halo	45S	3	29.67	2.08	29.00		
Degradation halo	5Q	3	10.33	0.58	10.00		
Degradation halo	8S	3	37.67	2.89	36.00		
Degradation halo	9LB	3	35.33	0.58	35.00		
Degradation halo	9M	3	34.67	0.58	35.00		

Figure no. 4.6: Non normally distributed data determined by Shapiro-Wilks's test and significant differences in the data of cellulase producing bacteria from the hindgut of *Uca rapax* in the wet season by Kruskal Wallis's test. Normal data ($p > 0.05$) and Non-normal data ($p < 0.05$). All the analyses were conducted in InfoStat® (InfoStat®, 2004) program.

Shapiro-Wilks (modified)

Variable	n	Mean	S.D.	W*	p(one tail)
Degradation Halo	30	18.73	12.74	0.79	<0.0001

Kruskal Wallis Test

Variable	Strains	N	Means	S.D.	Medians	H	p
Degradation Halo	17M	3	7.00	0.00	7.00	27.17	0.0011
Degradation Halo	20LB	3	43.67	1.53	44.00		
Degradation Halo	20M	3	7.33	0.58	7.00		
Degradation Halo	21S	3	30.00	1.00	30.00		
Degradation Halo	26S	3	22.00	1.00	22.00		
Degradation Halo	29M	3	22.67	0.58	23.00		
Degradation Halo	37M	3	31.33	2.31	30.00		
Degradation Halo	48M	3	7.17	0.29	7.00		
Degradation Halo	4M	3	8.33	0.58	8.00		
Degradation Halo	7M	3	7.83	0.29	8.00		

Figure no. 4.7: Non normally distributed data determined by Shapiro-Wilks's test and significant differences in the data of cellulase producing bacteria from the hindgut of *Uca rapax* in the dry season by Kruskal Wallis's test. Normal data ($p > 0.05$) and Non-normal data ($p < 0.05$). All the analyses were conducted in InfoStat® (InfoStat®, 2004) program.

Analysis of variance

Variable	N	R^2	Adj R^2	CV
Degradation halo	93	0.99	0.99	4.75

Analysis of variance table (Partial SS)

S.V.	SS	df	MS	F	p-value
Model.	9951.83	30	331.73	230.23	<0.0001
Strains	9951.83	30	331.73	230.23	<0.0001
Error	89.33	62	1.44		
Total	10041.16	92			

Test:Tukey Alpha:=0.05 LSD:=3.86941

Error: 1.4409 df: 62

Strains	Means	n	S.E.										
17Q	7.67	3	0.69	A									
23Q	8.00	3	0.69	A									
17LB	9.00	3	0.69	A									
11Q	9.00	3	0.69	A									
16Q	10.00	3	0.69	A									
18LB	10.00	3	0.69	A									
5Q	10.33	3	0.69	A									
33S	15.67	3	0.69		B								
10Q	18.00	3	0.69		B								
13Q	19.33	3	0.69		B								
3Q	24.33	3	0.69			C							
15LB	24.33	3	0.69			C							
37S	24.33	3	0.69			C							
38S	26.00	3	0.69			C	D						
31S	27.67	3	0.69			C	D	E					
45S	29.67	3	0.69				D	E	F				
20M	30.33	3	0.69					E	F				
41S	30.67	3	0.69					E	F	G			
39S	31.00	3	0.69					E	F	G	H		
32S	31.67	3	0.69						F	G	H	I	
19Q	31.67	3	0.69						F	G	H	I	
40S	32.00	3	0.69						F	G	H	I	J
28Q	32.33	3	0.69						F	G	H	I	J
27Q	34.33	3	0.69							G	H	I	J
9M	34.67	3	0.69								H	I	J
15S	35.00	3	0.69									I	J
9LB	35.33	3	0.69									I	J
22Q	35.67	3	0.69										J
8S	37.67	3	0.69										
28S	38.00	3	0.69										
14Q	40.33	3	0.69										

Means with a common letter are not significantly different (p<= 0.05)

Figure no. 4.8: Non parametrics ANOVA by Krustal Wallis's test. Multiple comparisons to determine significant differences among the cellulase producing bacterial strains by Tukey test in the wet season. All the analyses were conducted in InfoStat® (InfoStat®, 2004) program.

Analysis of variance

Variable	N	R^2	Adj R^2	CV
Degradation Halo	30	1.00	0.99	5.56

Analysis of variance table (Partial SS)

S.V.	SS	df	MS	F	p-value
Model.	4685.70	9	520.63	480.58	<0.0001
Strains	4685.70	9	520.63	480.58	<0.0001
Error	21.67	20	1.08		
Total	4707.37	29			

Test:Tukey Alpha:=0.05 LSD:=3.00936

Error: 1.0833 df: 20

Strains	Means	n	S.E.				
17M	7.00	3	0.60	A			
48M	7.17	3	0.60	A			
20M	7.33	3	0.60	A			
7M	7.83	3	0.60	A			
4M	8.33	3	0.60	A			
26S	22.00	3	0.60		B		
29M	22.67	3	0.60		B		
21S	30.00	3	0.60			C	
37M	31.33	3	0.60			C	
20LB	43.67	3	0.60				D

Means with a common letter are not significantly different (p<= 0.05)

Figure no. 4.9: Non parametrics ANOVA by Krustal Wallis's test. Multiple comparisons to determine significant differences among the cellulase producing bacterial strains by Tukey test in the dry season. All the analyses were conducted in InfoStat® (InfoStat®, 2004) program.

4.4 Discussion and Conclusion

Detritus is the main food source of fiddler crabs, which is mainly composed of organic matter (lignocellulose components) already in process of decomposition by an active microbial community. Total microbial biomass is never greater than 1.2% of the whole detrital mass, and in most cases is substantially less than 1%, which implies that detritivores organisms cannot rely solely on microorganisms as an energy source (Blum et al. 1988). We studied the degradation

capacity of cellulose of these microbial communities in both seasons and more than 80% were cellulases producers under laboratory conditions. The principal cellulose-degrader phylotypes in *Uca rapax* hindgut present during both seasons were Proteobacteria and Actinobacteria. The statistical analysis, especially the variance analyses, showed significant differences of the cellulose degradation capacity between bacterial strains suggesting that some strains have different enzymatic capabilities when degrading the cellulose. *Streptomyces, Paracoccus* and *Brachybacterium* were the cellulase-producing genera with the greater degradation capacity in both seasons. Despite *Vibrio* being always present in the hindgut, they showed low cellulose degradation capacity.

The Firmicutes (especially *Bacillus*) were excluded from the statistical analysis because they were also present in mangrove soil in both seasons. Nonetheless, we also analyzed the cellulose degradation capacity of *Bacillus* isolated from the hindgut of *Uca rapax* and more than 60% were positive cellulase producers. The capacity of selected *Bacillus* strains to produce and secrete large quantities (20–25 g/L) of extracellular enzymes (cellulases, chitosanases, pectate lyases, chitinases, proteases, lipases and levanase among others) has placed them among the most important industrial enzyme producers (Schallmey et al. 2004).

It was expected that the majority of the microflora would be able to degrade cellulose, as it is the main component in the detritus. We can confirm that bacterial communities were involved in the recycling of nutrients, and therefore nutritionally benefiting the fiddler crabs. Cellulase activity has been reported in many marine invertebrates, but it is not clear whether degradation of cellulose can be carried out by the fiddler crabs, through their associated microorganisms or both. One possibility is that cellulase degradation by microorganisms may occur in the soil prior to ingestion. According to Dall et al. (1983), in most crustaceans the food

spends only few hours in the digestive system and there is not the sufficient time for bacterial decomposition or digestion of carbohydrates occurs. However, cellulose activity makes an important contribution to the nutrition by converting cellulose into simpler carbohydrates prior to the ingestion of detritus by the fiddler crabs.

REFERENCES

Alavandi, S.1990. Relationship between heterotrophic bacteria and suspended particulate matter in the Arabian Sea. Ind. J. Mar. Sci. 30: 89-92.

Alongi, Daniel M. 1994. The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems. Hydrobiologia. 285: 19-32.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Beinlich, B. and H.O. von Hagen. 2006. Materials for a more stable subdivision of the genus *Uca leach*. Zoologische Mededelingen, 80(2): 9-32.

Benson DA, M. Cavanaugh, K. Clark, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell and E.W. Sayers. 2013. GenBank. Nucleic. Acids Res. 41: 36-42.

Bland, J. Martin and Douglas G. Altman. 1995. Multiple significance tests: the Bonferroni method. BMJ. 310: 170.

Blum LK, A.L. Mills, J.C. Zieman and R.T. Zieman. 1988. Abundance of bacteria and fungi in seagrass and mangrove detritus. Mar. Ecol. Prog. Ser. 42:73–78.

Brenner, D. J., N. R. Krieg, G. M. Garrity and J. D. Staley. 2005. Bergey's manual of systematic bacteriology. Springer Press.

Brown, Mark V. and John P. Bowman. 2001. A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). FEMS Microbiol. Ecol. 35: 267-275.

Castiglioni, Daniela da Silva and Maria Lucia Negreiros-Fransozo. 2004. Comparative analysis of the relative growth of *Uca rapax* (Smith) (Crustacea, Ocypodidae) from two mangroves in São Paulo, Brazil. Rev. Bras. Zool. 21(1): 137-144.

Colwell, R. K. 2009. EstimateS: Statistical estimation of species richness and shared species from samples, Version 8. User's Guide and Application published at: http://viceroy.eeb.uconn.edu/estimates.

Crane, J. 1975. Fiddler crabs of the world: ocypodidae: genus Uca. Princeton, NJ: Princeton University Press.

Dall, W., and D. J. W. Moriarty. 1983. Functional aspects of nutrition and digestion, p. 215-253. In L. Mantel (ed.), The Biology of Crustacea Volumen 5: Internal Anatomy and Physiologycal Regulation. Academic Press.

Dias ACF, Andreote FD, Dini-Andreote F, Lacava PT, Sa ALB, Melo IS, Azevedo JL, Araujo WL (2009) Diversity and biotechnological potential of culturable bacteria from Brazilian mangrove sediment. World J Microbiol Biotechnol. 25(7):1305–1311.

DRNA. Estado Libre Asociado de Puerto Rico; Departamento de Recursos Naturales y Ambientales. Plan De Manejo Para el Área De Planificación Especial Del Suroeste, Sector Boquerón; Programa de Manejo de la Zona Costanera de Puerto Rico. Noviembre, 2008.http://www.drna.gobierno.pr/oficinas/saux/secretaria-auxiliar-de-planificacion-integral/division-de-planificacion-de-recursos-terrestres/Plan%20de%20Manejo%20APE%20Suroeste%20Boqueron%20_Version%20PDF_.pdf.

Farmer, J. J., and F. W. Hickman-Brenner. 2006. The genera *Vibrio* and *Photobacterium*, p.508-561. In M. Dworkin et al. (ed), The Prokaryotes: A handbook on the biology of bacteria. Springer.

Genoni, Guilio P. 1991. Increased burrowing by fiddler crabs *Uca rapax* (Smith) (Decapoda: Ocypodidae) in response to low food supply. J. Exp. Mar. Biol. Ecol. 147(2): 267-285.

Ghosh, Abhrajyoti, Nirmalya Dey, Amit Bera, Amit Tiwari, KB Sathyaniranjan, Kalyan Chakrabarti and Dhrubajyoti Chattopadhyay. 2010. Culture independent molecular analysis of bacterial communities in the mangrove sediment of Sundarban, India. Saline Systems 6:1.

Gomes, Newton C.M., Daniel F.R., Cleary, Ricardo Calado and Rodrigo Costa. 2011. Mangrove bacterial richness. Communicative & Integrative Biology. 4(4): 419-423.

Gulmann, L. K. 2004. Gut-associated microbial symbionts of the marsh fiddler crab, *Uca pugnax*. Thesis PhD. Massachusetts Institute of Technology and Woods Hole Oceanography Institution. Online: https://darchive.mblwhoilibrary.org/handle/1912/1858.

Harris, J. M. 1993. The presence, nature, and role of gut microflora in aquatic invertebrates: A synthesis. Microb. Ecol. 25:195-231.

Harris, J. M., L. J. Seiderer, and M. I. Lucas. 1991. Gut microflora of two saltmarsh detritivore Thalassinid Prawns, Upogebia africana and Callianassa kraussi. Microb. Ecol. 21:277-296.

Hemmi, Jan M. and J. Zeil. 2003. Burrow surveillance in fiddler crabs. J. Exp. Biol. 206: 3935-3950.

Holgin, G., P. Vazquez, and Y. Bashan. 2001. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. Biol. Fertil. Soils. 33:265–278.

Holguin G, Guzman MA, Bashan Y (1992) Two new nitrogen fixing bacteria from the rhizosphere of mangrove trees: their isolation, identification and in vitro interaction with rhizosphere staphylococcus sp. FEMS Microbiol. 101:207–216.

Hong K, Gao AH, Xie QY, Gao H, Zhuang L, Lin HP, Yu HP, Li J, Yao XC, Goodfellow M, Ruan JS (2009) Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. Mar Drugs. 7:24–44.

InfoStat. 2009. InfoStat, versión 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina.

Justice, S., D. Hunstad, L. Cegelski and S. Hultgren. 2008. Morphological plasticity as a bacterial survival strategy. Nat. Rev. Microbiol. 6: 162-168.

Kasana, R. C., R. Salwan, H. Dhar, S. Dutt, and A. Gulati. 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. Curr. Microbiol. 57:503–507.

Kathiresan, K. and B. L. Bingham. 2001. Biology of mangroves and mangrove ecosystems. Adv. Mar. Biol. Vol. 40. Academic Press.

Kristensen, E. 2008. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. J. Sea Res. 59: 30–43.

Kruskal, William H. and W. Allen Wallis. 1952. Use of Ranks in One-Criterion Variance Analysis. J. Am. Stat. Assoc. 47(260): 583-621.

Lane, D. J. 1991. 16s/23S rRNA Sequencing, p. 115-175. In E. Stackebrandt, and M. Goodfellow (ed.), Nucleic Acid Techniques in Bacterial Systematics . John Wiley and Sons, Chichester, United Kingdom.

Lau, W. W. Y., P. A. Jumars, and E. V. Armbrust. 2002. Genetic diversity of attached bacteria in the hindgut of the deposit-feeding shrimp *Neotrypaea* (formerly Callianassa) *californiensis* (decapoda: Thalassinidae). Microb. Ecol. 43: 455-466.

Lennon, Jay T., Zachary T. Aanderud, B. K. Lehmkuhl and Donald R. Schoolmaster, Jr. 2012. Mapping the niche space of soil microorganisms using taxonomy and traits. Ecology. 93(8): 1867–1879.

Liang, Jun-Bin, Yue-Qin Chen, Chong-Yu Lan, Nora F. Y. Tam, Qi-Jie Zan and Li-Nan Huang. 2007. Recovery of novel bacterial diversity from mangrove sediment. Mar. Biol. 150: 739–747.

Liao, Pei-Chun, Bing-Hong Huang and Shong Huang. 2007. Microbial Community Composition of the Danshui River Estuary of Northern Taiwan and the Practicality of the Phylogenetic Method in Microbial Barcoding. Microbial Composition and Practicality of the Phylogenetic Method. 54: 497–507.

Lozupone, C. and R. Knight. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71(12): 8228-8235.

Lugo, Ariel E., Ernesto Medina, Elvira Cuevas, Gilberto Cintrón, Eddie N. Laboy Nieves and Yara Schäeffer Novelli. 2007. Ecophysiology of a Mangrove Forest in Jobos Bay, Puerto Rico. Caribb. J. Sci. 43(2): 200-219.

McCarthy, A.J. 1987. Lignocellulose-degrading actinomycetes. FEMS Microbiol. Rev. 46:145-163.

Meziane, T., M. C. Sanabe, and M. Tsuchiya. 2002. Role of fiddler crabs of a subtropical intertidal flat on the fate of sedimentary fatty acids. J. Exp. Mar. Biol. Ecol. 270:191-201.

Microsoft® Excel®.2010. (14.0.6126.5003) SP 1 MSO (14.0.6129.5000) Part of Microsoft Office Home and Student 2010 © Microsoft Corporation.

Ng, P. K. L, D. Guinot and P. Davie. 2008. Systema Brachyurorum: Part I. An annotated checklist of extant Brachyuran crabs of the world. The raffles bulletin of zoology. 17: 1-286.

Nyholm, Spencer V., Eric V. Stabb, Edward G. Ruby and Margaret J. McFall-Ngai. 2000. Establishment of an animal– bacterial association: Recruiting symbiotic vibrios from the environment. PNAS. 97(18): 10231-10235.

Nyholm, Spencer V. and Margaret J.McFall-Ngai. 2004. The Winnowing: Establishing

The Squid–Vibrio Symbiosis. Nature. 2: 632-642.

Nyman, J, A., R. J. Walters, R. D. Delaune, and W. H. Patrick Jr. 2006. Marsh vertical accretion via vegetative growth. Estuarine Coastal and Shelf Science. 69: 370-380.

Odum, W.E. and E.J. Heald. 1975. The detritus-based food web of an estuarine mangrove community. In: Ronin LT (ed) Estuarine research. Academic Press.

Pitts, G. Y. and G. T. Cowley. 1974. Mycoflora of the habitat and midgut of the fiddler crab, *Uca pugilator*. Mycologia. 66: 669-675.

Plante, C. J., P. A. Jumars, and J. A. Baross. 1990. Digestive associations between marine detritivores and bacteria. Annu. Rev. Ecol. Syst. 21: 93-127.

Pointing, S. B. 1999. Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. Fungal Divers. 2: 17-33.

Polidoro, B. A., K. E. Carpenter, L. Collins, N. C. Duke, A. M. Ellison, J. C. Ellison, E. J. Farnsworth, E. S. Fernando, K. Kathiresan, N. E. Koedam, S. R. Livingstone, T. Miyagi, G. E. Moore, V. N. Nam, J. E. Ong, J. H. Primavera, S. G. Salmo, J. C. Sanciangco, S. Sukardjo, Y. Wang, and J. W. H. Yong. 2010. The loss of species: mangrove extinction risk and geographic areas of global concern. PLoS ONE 5:4.

Rivera, Manuel y Manul J. Velez, Jr. 1982. Observaciones preliminaries sobre los cangrejos del género *Uca* (Decapoda:Ocypodidae) en Puerto Rico. Noveno simposio de recursos naturales del Departamento de Recursos Naturales.

Robertson, J. R., and S. Y. Newell. 1982. Experimental studies of particle ingestion by the sand fiddler crab *Uca pugilator* (Bosc). J. Exp. Mar. Biol. Ecol. 59:1-21.

Saleem-Khan, A., M. Sheik Ali and I. Juned Ahmed Baig. 2009. Halophilic (aerobic) bacterial growth rate of mangrove ecosystem. J. Environ. Biol. 30(5): 705-707.

Santos, Henrique F., Flávia L. Carmo, Jorge E. S. Paes, Alexandre S. Rosado and Raquel S. Peixoto. 2011. Bioremediation of Mangroves Impacted by Petroleum. Water Air Soil Poll. 216(1-4): 329-350.

Santos, Henrique F., Juliano Carvalho Cury, Flavia Lima do Carmo, Adriana Lopes dos Santos, James Tiedje, Jan Dirk van Elsas, Alexandre Soares Rosado and Raquel Silva Peixoto. 2011. Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: bacterial proxies for oil pollution. PLoS ONE 6(3): e16943.

Schallmey, Marcus, Ajay Singh, and Owen P. Ward. 2004. Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol. 50: 1–17.

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology. 88:1386–1394.

Sequencher® version 5.0 sequence analysis software. Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com

Shannon, C. and W. Weaver.1949. The mathematical theory of communication. University of Illinois Press.

Shapiro, S. S. and M. B. Wilk. 1965. An Analysis of Variance Test for Normality (Complete Samples). Biometrika Trust. 52(3-4): 591-611.

Simpson, E.H. 1949. Measurements of diversity. Nature 163: 688.

Tabao NC, Moasalud RG. 2010. Characterisation and identification of high cellulose-producing bacterial strains from Philippine mangroves. Philipp J System Biol. 4:13–20.

Taruma K., D. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular Evolucionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. 28: 2731-2739.

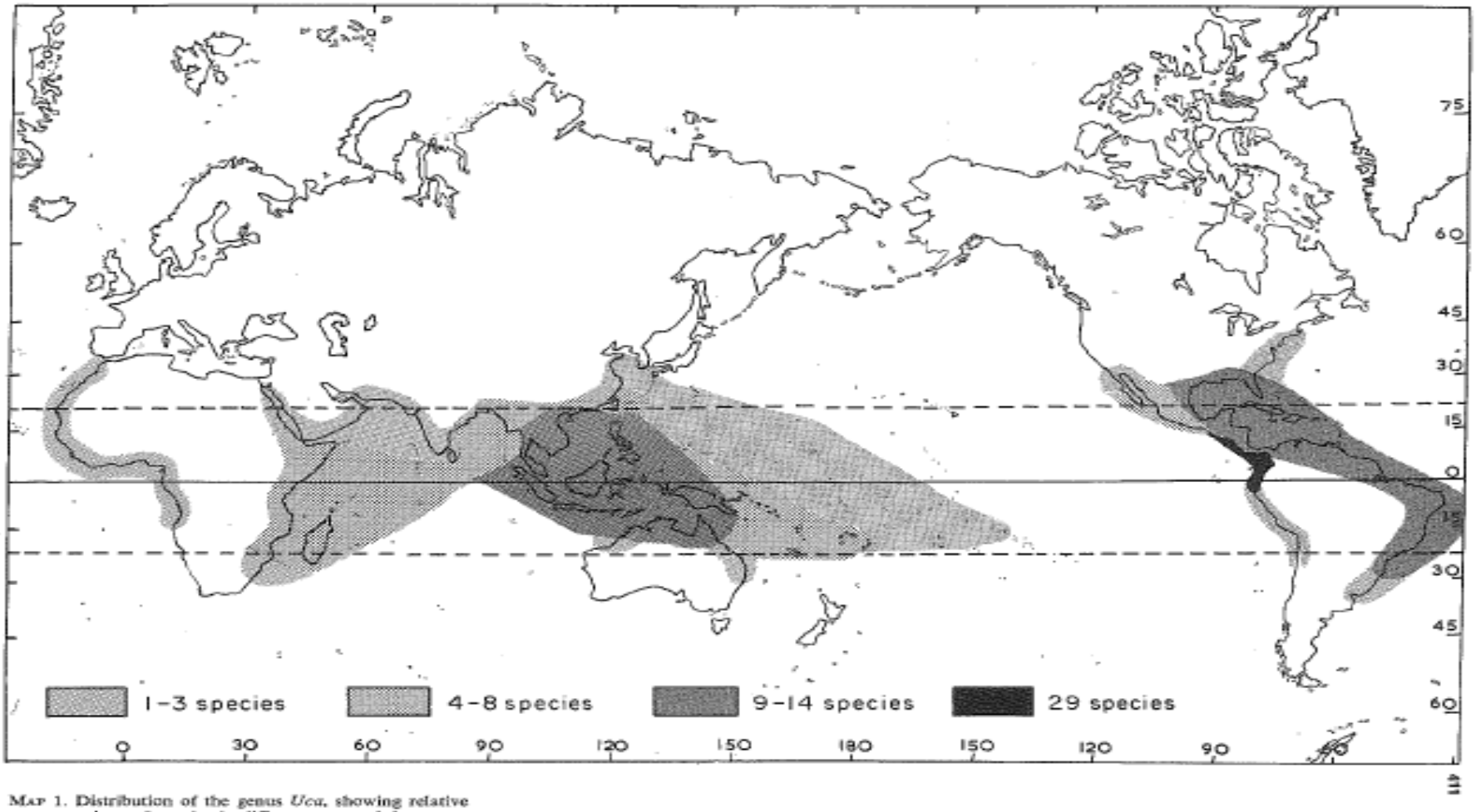
Thatoi, Hrudayanath, Bikash Chandra Behera, Rashmi Ranjan Mishra and Sushil Kumar Dutta. 2013. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. Ann Microbiol. 63:1–19.

Urbanczyk, Henryk, Jennifer C. Ast and Paul V. Dunlap. 2010. Phylogeny, genomics, and symbiosis of Photobacterium. FEMS Microbiol Rev. 35: 324-342.

Zeil, J., J. M. Hemmi, and P.R. Y. Backwell. 2006. Fiddler crabs: Quick guide. Curr. Biol. 16: 2.

Zucchi, T. D., A. S. Guidolin, and F. L. Cônsoli. 2009. Isolation and characterization of actinobacteria ectosymbionts from Acromyrmex subterraneus brunneus (Hymenoptera, Formicidae) Microbiol. Res. doi:10.1016/j.micres.2010.01.009.

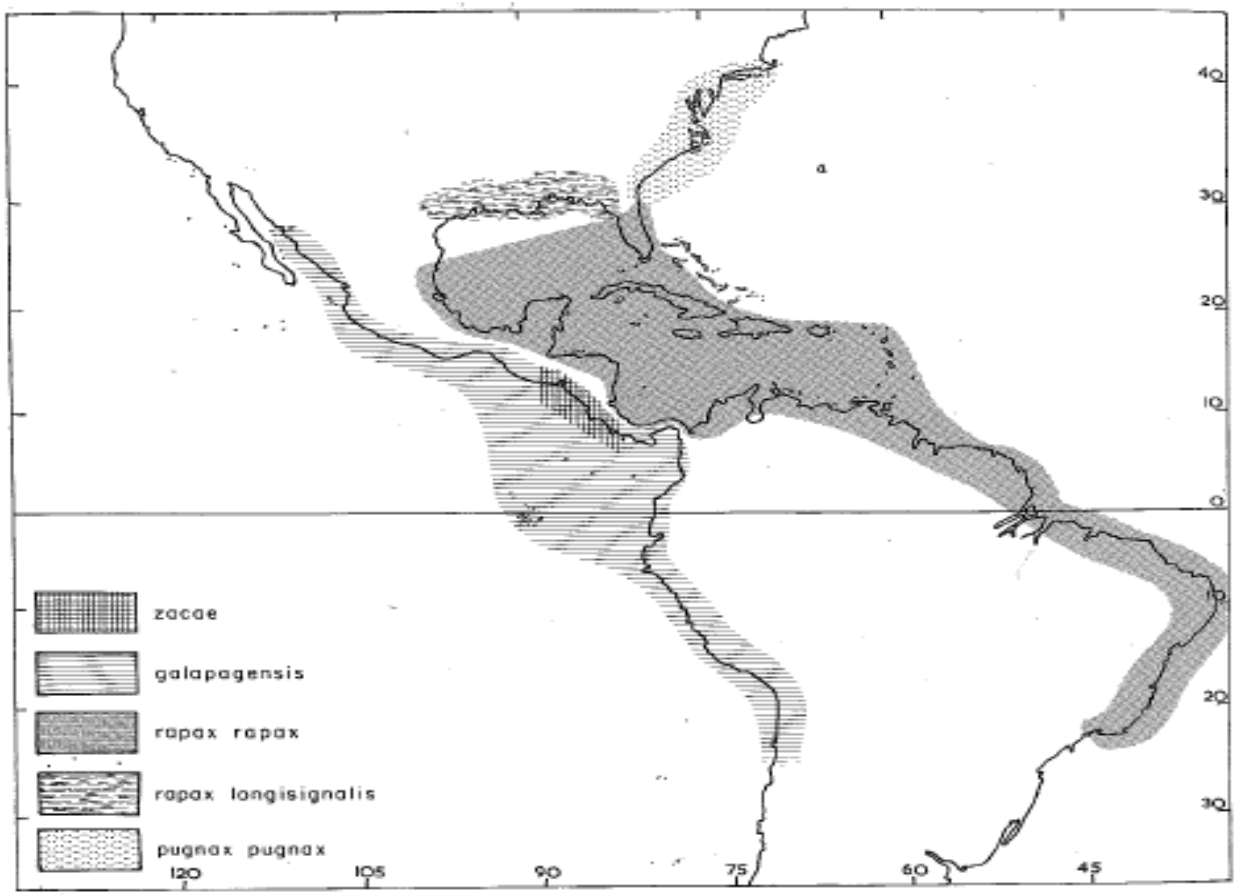
Appendix A: Global distribution of the genus *Uca.*



MAP 1. Distribution of the genus *Uca*, showing relative concentrations of species in different parts of the range. See p. 409ff.

Craine, (1975)

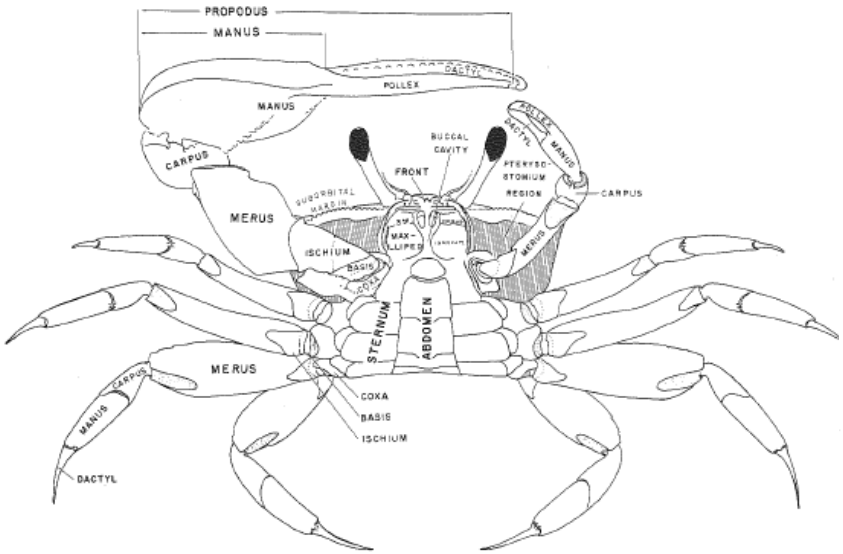
Appendix B: Distribution of subgenus *Minuca*; *Uca rapax.*

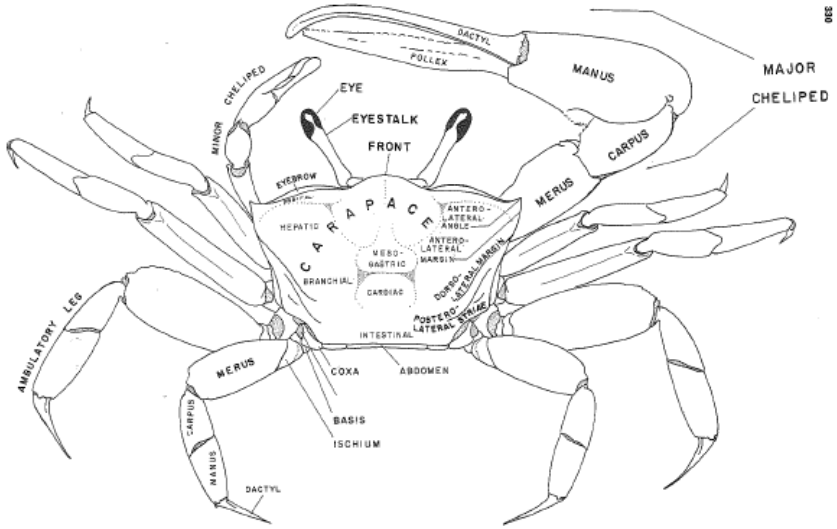


MAP 14. Distribution of the subgenus *Minuca* (concluded); for the distribution of (*Minuca*) *pugnax virens*, see Map 10. (General explanation: p. 409.)

Craine, (1975)

Appendix C: Ventral and dorsal view of fiddler crabs.





Craine, (1975)

Appendix D: Guaniquilla mangrove in "Bosque Estatal de Boquerón"; Cabo Rojo, Puerto Rico.

DRNA, (2008)

Appendix E: Bacteria isolated from mangrove soil from Guaquinilla region, Boquerón, Puerto Rico (Wet season).

DNA/PCR Codes	Plates Codes	Accesion Number	Identification	Phylum	Class	Query Covarage / Max Identity	Source	Color	Shape	Border	Elevation
1	BQ SOIL M-2	GU397426.2	*Halomonas smyrnensis*	Proteobacteria	γ	22% / 98%	Saltern area	white	punctiform	lobate	flat
2	BQ SOIL M-4	JN791326.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	93% / 99%	marine sediment	yellow	circular	entire	convex
3	BQ SOIL M-2(1)	EU124555.1	*Bacillus megaterium*	Firmicutes	Bacilli	98% / 99%	industrial site	white	circular	lobate	flat
4	BQ SOIL M-13	JN791328.1	*Microbulbifer sp*	Proteobacteria	γ	91% / 93%	marine sediment	yellow	circular	entire	flat
5	BQ SOIL M-14	FJ527419.1	*Microbulbifer sp*	Proteobacteria	γ	98% / 99%	Soil	brown	circular	entire	convex
6	BQ SOIL M-15	HM233989.1	*Bacillus sp.*	Firmicutes	Bacilli	97% / 99%	Roots	cream	filamentous	filamentous	convex
7	BQ SOIL M-17	EF612310	*Rhodococcus sp.*	Actinobacteria	Actinobacteridae	52% / 87%	soil	orampe	circular	entire	convex
8	BQ SOIL M-19	JN315890.1	*Staphylococcus arlettae*	Firmicutes	Bacilli	97% / 90%	N/A	yellow	punctiform	undulate	flat
9	BQ SOIL M-22(1)	JN315890.1	*Staphylococcus arlettae*	Firmicutes	Bacilli	97% / 99%	prawn	white	circular	entire	flat
11	BQ SOIL M-27	EU868848.1	*Halomonas sp.*	Proteobacteria	γ	97% / 98%	hypersaline pond	brown	rhizoid	filamentous	raised
12	BQ SOIL M-30(2)(1)	AJ577277.1	*Bacillus cereus*	Firmicutes	Bacilli	87% / 96%	N/A	white	punctiform	lobate	flat
13	BQ SOIL M-30(2)(2)	JQ236629.1	*Lysinibacillus sp.*	Firmicutes	Bacilli	94% / 100%	plants	clear	rhizoid	amentous/lob	flat
14	BQ SOIL M-32a(2)(1)	JF970585.1	*Lysinibacillus fusiformis*	Firmicutes	Bacilli	100% / 99%	continental ice	cream	circular	filamentous	convex
15	BQ SOIL M-35	GU968467.1	*Oceanimonas sp.*	Proteobacteria	γ	96% / 99%	coastal soil	white	circular	entire	flat
16	BQ SOIL M-38(1)(1)	HQ202555.1	*Bacillus megaterium*	Firmicutes	Bacilli	98% / 95%	Tobacco Rhizosphere	white	circular	entire	flat
17	BQ SOIL M-38(2)(1)	EU912460.1	*Bacillus sp.*	Firmicutes	Bacilli	70% / 84%	pine	clear	punctiform	undulate	flat
18	BQ SOIL M-42	GU980766.1	*Oceanimonas smirnovii*	Proteobacteria	γ	59% / 98%	industrial area	brown	circular	entire/curled	convex
19	BQ SOIL M-50	FM163605.1	*Oceanimonas doudoroffii*	Proteobacteria	γ	48% / 96%	agricultural soils	brown	circular	lobate/curled	convex
20	BQ SOIL M-53	AM990844.1	*Halomonas sp.*	Proteobacteria	γ	94% / 99%	coastal soil	brown	circular	entire	convex
21	BQ SOIL M-55(1)	GU968467.1	*Oceanimonas sp*	Proteobacteria	γ	57% / 99%	coastal soil	brown	circular	curled	flat
22	BQ SOIL M-60(2)	HM068886.1	*Serinicoccus chungangensis*	Actinobacteria	Actinobacteridae	97% / 99%	tidal flat	white	circular	undulate	flat
23	BQ SOIL M-68	JF830616.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	92% / 98%	seagrasses	yellow	punctiform	entire	flat
24	BQ SOIL M-75	JQ579631.1	*Bacillus megaterium*	Firmicutes	Bacilli	98% / 99%	peanut	cream	circular	lobate	flat
25	BQ SOIL M-76	NR_044538.1	*Bacillus korlensis*	Firmicutes	Bacilli	98% / 99%	sand soil	gray	filamentous	lobate	flat
26	BQ SOIL M-77	AB674956.1	*Demequina sp.*	Actinobacteria	Actinobacteridae	94% / 99%	sea sediment	white	circular	entire	convex
27	BQ SOIL M-85(2)	JN128237.1	*Staphylococcus cohnii*	Firmicutes	Bacilli	97% / 99%	Marine Sponge	white	circular	entire	flat
28	BQ SOIL M-87	NR_044366.1	*Nocardia altamirensis*	Actinobacteria	Actinobacteridae	30% / 89%	N/A	pink	circular	entire	flat
29	BQ SOIL M-92(1)	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	96% / 99%	seagrasses	yellow	circular	filamentous	convex
30	BQ SOIL M-97(2)	JQ398853.1	*Bacillus subtilis*	Firmicutes	Bacilli	98% / 99%	roots of lemon grass	white	circular	lobate	flat
1	BQ SOIL LB-1	HM061611.1	*Bacillus megaterium*	Firmicutes	Bacilli	57% / 96%	N/A	cream	punctiform	undulate	flat
2	BQ SOIL LB-3	JF304284.1	*Bacillus humi*	Firmicutes	Bacilli	100% / 99%	N/A	white	circular	entire	convex
3	BQ SOIL LB-4	JN942146.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 99%	Oil	yellow	irregular	curled	flat
4	BQ SOIL LB-5(1)	JN942152.1	*Gordonia sp.*	Actinobacteria	Actinobacteridae	97% / 99%	surface water	orange	circular	entire	flat
5	BQ SOIL LB-6	JN791326.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	93% / 99%	marine sediment	yellow	irregular	filamentous	convex
6	BQ SOIL LB-7	JQ348902.1	*Bacillus pumilus*	Firmicutes	Bacilli	97% / 99%	Soil	orange	irregular	curled	flat
7	BQ SOIL LB-8	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 99%	surface water	yellow	irregular	undulate	flat
8	BQ SOIL LB-11	HQ154558.1	*Staphylococcus sciuri*	Firmicutes	Bacilli	98% / 99%	midgut	yellow	irregular	entire	flat
9	BQ SOIL LB-15	HQ176466.1	*Nitratireductor sp.*	Proteobacteria	α	96% / 99%	diatom	white	irregular	undulate	flat
10	BQ SOIL LB-17(1)	JF820115.1	*Bacillus sp.*	Firmicutes	Bacilli	99% / 99%	Soil	white	irregular	curled	crateriform
11	BQ SOIL LB-18(2)(2)	AY277554.1	*Gordonia rubripertinctus*	Actinobacteria	Actinobacteridae	97% / 100%	hypogean environments	orange	irregular	undulate	convex
12	BQ SOIL LB-18(3)(1)	AY277554.1	*Gordonia rubripertinctus*	Actinobacteria	Actinobacteridae	97% / 100%	hypogean environments	orange	circular	entire	raised
13	BQ SOIL LB-19	GQ284523.1	*Bacillus sp.*	Firmicutes	Bacilli	99% / 99%	mangrove sediment	clear	circular	erose	flat
14	BQ SOIL LB-20	EU741242.1	*Gordonia sp.*	Actinobacteria	Actinobacteridae	97% / 98%	beach sand	pink	circular	entire	convex
15	BQ SOIL LB-23	HQ696430.1	*Bacillus safensis*	Firmicutes	Bacilli	98% / 99%	stands	white	irregular	undulate	flat
16	BQ SOIL LB-26	NR_028823.1	*Gordonia sinesedis*	Actinobacteria	Actinobacteridae	93% / 99%	Soil	pink	irregular	entire	flat
17	BQ SOIL LB-27	JF683607.1	*Bacillus megaterium*	Firmicutes	Bacilli	98% / 99%	Soil	white	circular	erose	flat
18	BQ SOIL LB-29	JQ291586.1	*Cellulosimicrobium funkei*	Actinobacteria	Actinobacteridae	97% / 100%	larvae gut	yellow	circular	entire	flat
19	BQ SOIL LB-32	NR_028823.1	*Gordonia sinesedis*	Actinobacteria	Actinobacteridae	97% / 98%	Soil	yellow	circular	entire	flat
20	BQ SOIL LB-33	HM068886.1	*Serinicoccus chungangensis*	Actinobacteria	Actinobacteridae	98% / 99%	tidal flat sediment	yellow	circular	entire	flat
21	BQ SOIL LB-36	JF917312.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 99%	seagrasses	yellow	irregular	curled	flat
22	BQ SOIL LB-37	JN128279.1	*Microbacterium esteraromaticum*	Actinobacteria	Actinobacteridae	93% / 92%	marine sponge	yellow	circular	entire	flat
23	BQ SOIL LB-42	AM778450.1	*Microbacterium jejuense*	Actinobacteria	Actinobacteridae	76% / 94%	seawater	yellow	circular	entire	convex
24	BQ SOIL LB-43	GU220451.1	*Gordonia sp.*	Actinobacteria	Actinobacteridae	94% / 85%	soil	brown	filamentous	filamentous	umbonate
25	BQ SOIL LB-46	JN942152.1	*Gordonia sp.*	Actinobacteria	Actinobacteridae	97% / 99%	oil-contaminated surface water	pink	circular	undulate	raised

Appendix E: Continuation

1	BQ SOIL Q-3	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	81% / 96%	Seagrasses	brown	circular	entire	convex
2	BQ SOIL Q-6	JN700144.1	*Bacillus cereus*	Firmicutes	Bacilli	79% / 98%	Plants	white	circular	filamentous	flat
3	BQ SOIL Q-8	FJ267560.1	*Novosphingobium sp.*	Proteobacteria	α	47% / 97%	N/A	clear	circular	entire	convex
4	BQ SOIL Q-11(1)	FJ938159.1	*Sphingomonas sp.*	Proteobacteria	α	65% / 98%	lake sediment	orange	irregular	lobate	convex
5	BQ SOIL Q-11(2)	HQ610620.1	*Lysinibacillus fusiformis*	Firmicutes	Bacilli	78% / 95%	vermicompos	cream	circular	erose	flat
6	BQ SOIL Q-7(2)(2)	JN627177.1	*Mycobacterium poriferae*	Actinobacteria	Actinobacteridae	97% / 99%	Sea soft coral	yellow	circular	entire	flat
7	BQ SOIL Q-14	JN426847.1	*Algoriphagus sp.*	Bacteroidetes	Cytophagia	85% / 94%	mangrove sediment	cream	circular	entire	convex
8	BQ SOIL Q-17	HM130543.1	*Staphylococcus pasteuri*	Firmicutes	Bacilli	88% / 95%	fermented vegetable	orange	circular	entire	flat
9	BQ SOIL Q-19(3)(3)	JQ396542.1	*Sphingomonas sp.*	Proteobacteria	α	76% / 86%	arctic rhizosphere	yellow	punctiform	amentous/ent	convex
10	BQ SOIL Q-20a	JN791326.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	93% / 99%	marine sediment	yellow	punctiform	entire	flat
12	BQ SOIL Q-21	GU451185.1	*Microbacteriaceae bacterium*	Actinobacteria	Actinobacteridae	98% / 99%	adult gut	yellow	circular	entire	convex
13	BQ SOIL Q-24a(1)	HM210420.1	*Mycobacterium sp.*	Actinobacteria	Actinobacteridae	97% / 99%	marine sponge	yellow	punctiform	entire	flat
14	BQ SOIL Q-24a(4)	JN942138.1	*Bacillus sp.*	Firmicutes	Bacilli	97% / 99%	Crude-oil	clear	circular	filamentous	umbonate
15	BQ SOIL Q-24b	JQ229800.1	*Brevibacillus borstelensis*	Firmicutes	Bacilli	98% / 99%	Rice	yellow	circular	entire	convex
16	BQ SOIL Q-27(1)	NR_044109.1	*Aeromicrobium flavum*	Actinobacteria	Actinobacteridae	94% / 99%	Soil	yellow	circular	entire	convex
17	BQ SOIL Q-27(2)	JN791326.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	93% / 99%	marine sediment	yellow	punctiform	filamentous	flat
18	BQ SOIL Q-27(3)	JF753455.1	*Cellulomonas sp.*	Actinobacteria	Actinobacteridae	75% / 88%	Zea species seed	brown	circular	entire	flat
19	BQ SOIL Q-29(1)	EU834248.1	*Cellulomonas denverensis*	Actinobacteria	Actinobacteridae	97% / 82%	Soil	white	punctiform	erose	flat
20	BQ SOIL Q-30	EU236753.1	*Curtobacterium sp.*	Actinobacteria	Actinobacteridae	47% / 97%	Plants	yellow	punctiform	entire	convex
21	BQ SOIL Q-31	AM990831.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 98%	sea water	yellow	circular	entire	flat
22	BQ SOIL Q-35(1)	DQ350882.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	99% / 99%	garden soil	brown	irregular	lobate	convex
23	BQ SOIL Q-35(2)	HM011217.1	*Mycobacterium bacteremicum*	Actinobacteria	Actinobacteridae	97% / 99%	N/A	white	rhizoid	entire	flat
24	BQ SOIL Q-39	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 84%	oil-contaminated surface water	yellow	circular	entire	flat
25	BQ SOIL Q-46	HQ622514.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	94% / 99%	Ocean deep sea	yellow	circular	entire	convex
26	BQ SOIL Q-48	JN942134.1	*Gordonia sp.*	Actinobacteria	Actinobacteridae	93% / 100%	crude oil-contaminated surface water	orange	irregular	undulate	convex
27	BQ SOIL Q-49(2)	JN791326.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	93 % / 99%	marine sediment	yellow	circular	amentous/cur	convex
28	BQ SOIL Q-50	EU834283.1	*Microbacterium kitamiense*	Actinobacteria	Actinobacteridae	42% / 82%	Soil	yellow	circular	entire	flat
29	BQ SOIL Q-51(1)	FJ905295	*Rhodococcus sp.*	Actinobacteria	Actinobacteridae	74% / 85%	N/A	orange	circular	entire	flat
1	BQ SOIL S-2(1)(1)(1)	JF820119.1	*Bacillus sp.*	Firmicutes	Bacilli	99% / 99%	Gas Field Soil	clear	circular	lobate	flat
2	BQ SOIL S-3(1)	JN999852.1	*Bacillus megaterium*	Firmicutes	Bacilli	96% / 100%	agricultural soil	cream	circular	entire	flat
3	BQ SOIL S-3(2)	GU217715.1	*Planococcus sp.*	Firmicutes	Bacilli	28% / 87%	mud volcano	white	circular	entire	flat
4	BQ SOIL S-4	GQ924938.1	*Mycobacterium monacense*	Actinobacteria	Actinobacteridae	97% / 99%	sputum	yellow	irregular	lobate	convex
5	BQ SOIL S-7(2)(1)	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 99%	oil-contaminated surface water	yellow	circular	entire	flat
6	BQ SOIL S-7(2)(2)	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	93% / 99%	seagrass	yellow	circular	entire	convex
7	BQ SOIL S-9	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	93% / 99%	seagrass	clear	irregular	lobate	flat
8	BQ SOIL S-14	HQ219958.1	*Microbacterium hydrocarbonoxydans*	Actinobacteria	Actinobacteridae	97% / 99%	roots	yellow	circular	undulate	convex
9	BQ SOIL S-15(2)	GQ496666.1	*Cellulosimicrobium cellulans*	Actinobacteria	Actinobacteridae	97% / 100%	maize	yellow	circular	amentous/cur	umbonate
10	BQ SOIL S-16	JN942152.1	*Gordonia sp.*	Actinobacteria	Actinobacteridae	97% / 99%	oil-contaminated surface water	orange	circular	entire	flat
11	BQ SOIL S-17	HM068886.1	*Serinicoccus chungangensis*	Actinobacteria	Actinobacteridae	97% / 99%	sediment	yellow	circular	entire	flat
12	BQ SOIL S-18(1)	JQ236812.1	*Bacillus sp.*	Firmicutes	Bacilli	52% / 80%	N/A	white	circular	erose	flat
13	BQ SOIL S-19(1)(1)	HQ219958.1	*Microbacterium hydrocarbonoxydans*	Actinobacteria	Actinobacteridae	97% / 99%	roots	yellow	circular	entire	flat
14	BQ SOIL S-24	JN627169.1	*Gordonia lacunae*	Actinobacteria	Actinobacteridae	93% / 100%	soft coral	orange	circular	entire	convex
15	BQ SOIL S-27	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	97% / 99%	seagrass	yellow	filamentous	filamentous	flat
16	BQ SOIL S-28	EF540451.1	*Agrococcus sp.*	Actinobacteria	Actinobacteridae	97% / 99%	solid waste	yellow	circular	entire	flat
17	BQ SOIL S-30	FN668009.1	*Bacillus megaterium*	Firmicutes	Bacilli	79% / 78%	mycorrhizal fungi	orange/white	irregular	undulate	convex
18	BQ SOIL S-31(2)(2)(2)(2)(2	HQ202555.1	*Bacillus megaterium*	Firmicutes	Bacilli	98% / 99%	soil	white	irregular	lobate	flat
19	BQ SOIL S-33	JQ236629.1	*Lysinibacillus sp.*	Firmicutes	Bacilli	93% / 99%	ethnomedicinal plants	yellow	circular	entire	convex
20	BQ SOIL S-36	JN794602.1	*Staphylococcus sp.*	Firmicutes	Bacilli	97% / 99%	manure	clear	circular	erose	flat
21	BQ SOIL S-37	JQ773352.1	*Rhodococcus sp.*	Actinobacteria	Actinobacteridae	95% / 99%	industrial wastewater	orange/white	circular	undulate	raised
22	BQ SOIL S-41	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	93% / 99%	seagrass	yellow	punctiform	entire	flat
23	BQ SOIL S-43	AB552874.1	*Microbacterium sp*	Actinobacteria	Actinobacteridae	97% / 99%	Algal-bacterial consortia	clear	circular	entire	flat
24	BQ SOIL S-44(2)(1)(2)	HQ231222.1	*Bacillus sp.*	Firmicutes	Bacilli	99% / 100%	soil	white	irregular	amentous/lob	flat

Appendix F: Bacteria isolated from mangrove soil from Guaquinilla region, Boquerón, Puerto Rico (Dry season).

DNA/PCR Codes	Plates Codes	Accesion Number	Identification	Phylum	Class	Query Coverage / Max Identity	Source	Color	Shape	Border	Elevation
2	BQSOILII M-2	FJ232508.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/99%	salterns	yellow	circular	erose	crateriform
3	BQSOILII M-5	FJ527419.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	forest soil	cream	circular	undulate	flat
4	BQSOILII M-6	FR822388.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	industrial contaminant	white	circular	erose	flat
5	BQSOILII M-7	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	cream	circular	filamentous	flat
6	BQSOILII M-8	JN791286.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	marine sediment	cream	circular	entire	flat
8	BQSOILII M-11	FJ601906.1	*Bacillus thuringiensis*	Firmicutes	Bacilli	100%/97%	wastewater sludge	white	circular	mentous/cur	flat
9	BQSOILII M-12	HM044219.1	*Bacillus aquimaris*	Firmicutes	Bacilli	100%/100%	Sinularia sp. (soft coral)	pink	circular	entire	flat
10	BQSOILII M-13	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	cream	irregular	lobate	flat
11	BQSOILII M-14	EU399548.1	*Marinobacterium sp.*	Proteobacteria	γ	100%/99%	marine sediment	cream	circular	undulate	flat
12	BQSOILII M-16	FJ386525.1	*Halobacillus sp.*	Firmicutes	Bacilli	99%/99%	salt lake	yellow	circular	curled	flat
13	BQSOILII M-17	NR_044794.1	*Microbulbifer hydrolyticus*	Proteobacteria	γ	100%/99%	lignin-rich pulp mill waste	cream	circular	undulate	flat
14	BQSOILII M-18	DQ448750.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	marine sediment	clear	circular	undulate	flat
15	BQSOILII M-19	FJ386525.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/99%	salt lake	cream	circular	entire	flat
16	BQSOILII M-20	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	orange	circular	emtire	umbonate
17	BQSOILII M-21	GQ304894.1	*Terribacillus aidingensis*	Firmicutes	Bacilli	100%/99%	saline lakes	clear	circular	entire	flat
18	BQSOILII M-22	GU223379.1	*Marinobacter sp.*	Proteobacteria	γ	100%/99%	ocean water	yellow	circular	undulate	flat
20	BQSOILII M-24	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	brown	circular	entire	flat
21	BQSOILII M-25	JX077093.1	*Bacillus cereus*	Firmicutes	Bacilli	100%/100%	fresh leaf	white	circular	filamentous	flat
22	BQSOILII M-26	JQ030912.1	*Bacillus aquimaris*	Firmicutes	Bacilli	100%/100%	ship dismantling zone sediment	cream	circular	entire	flat
23	BQSOILII M-27	HQ683724.1	*Halobacillus sp*	Firmicutes	Bacilli	100%/100%	salt lake	cream	circular	entire	umbonate
24	BQSOILII M-28	HQ683724.1	*Halobacillus sp*	Firmicutes	Bacilli	100%/100%	salt lake	cream	circular	entire	flat
25	BQSOILII M-29	EU143349.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	black sand	cream	circular	entire	convex
26	BQSOILII M-31	JN791286.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	marine sediment	cream	irregular	entire	convex
27	BQSOILII M-32(2)	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	99%/100%	salt lake	cream	circular	entire	flat
28	BQSOILII M-33	JQ030912.1	*Bacillus aquimaris*	Firmicutes	Bacilli	100%/100%	ship dismantling zone sediment	cream	circular	entire	flat
30	BQSOILII M-36	AJ829714.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	polluted aquifer	clear	circular	entire	flat
31	BQSOILII M-37	FJ386525.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/99%	salt lake	yellow	circular	erose	convex
32	BQSOILII M-42	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	yellow	circular	entire	convex
33	BQSOILII M-44	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	cream	circular	entire	flat
34	BQSOILII M-47	FJ441060.1	*Bacillus sp.*	Firmicutes	Bacilli	99%/99%	leaf	cream	circular	undulate	flat
36	BQSOILII M-51	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	cream	circular	undulate	flat
38	BQSOILII M-53	FJ386525.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	yellow	circular	entire	flat
39	BQSOILII M-55	JQ690689.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	hypersaline oil reservoir	cream	circular	entire	flat
41	BQSOILII M-58	JX077093.1	*Bacillus cereus*	Firmicutes	Bacilli	100%/100%	fresh leaf	white	circular	filamentous	raised
42	BQSOILII M-59	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	white	circular	lobate	flat
43	BQSOILII M-62	AY967717.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	coastal sea	clear	circular	entire	flat
45	BQSOILII M-65	GQ118708.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	sediment microbial mat	cream	circular	entire	convex
47	BQSOILII M-67	FJ444973.1	*Halobacillus trueperi*	Firmicutes	Bacilli	100%/99%	Saltern	yellow	circular	undulate	convex
48	BQSOILII M-68	AJ829714.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	aquifer	white	circular	entire	flat
49	BQSOILII M-69	GQ478405.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	Digestive Tract of Anas platyrhynchos (mallard)	yellow	circular	entire	flat
51	BQSOILII M-73	JQ799107.1	*Bacillus vietnamensis*	Firmicutes	Bacilli	100%/100%	tropical marine sediment	orange	circular	entire	convex
52	BQSOILII M-74	JF719277.1	*Ruegeria sp.*	Proteobacteria	α	100%/98%	ocean water	cream	circular	entire	convex
53	BQSOILII M-78	JN791328.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	marine sediment	cream	circular	entire	convex
54	BQSOILII M-79	JQ687116.1	*Terribacillus sp.*	Firmicutes	Bacilli	98%/79%	fresh water	white	circular	undulate	flat
55	BQSOILII M-80	HQ683724.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	orange	circular	entire	umbonate
56	BQSOILII M-81	JQ695932.1	*Bacillus firmus*	Firmicutes	Bacilli	100%/100%	rice rhizoshere	cream	circular	entire	flat
57	BQSOILII M-82	FJ386520.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/99%	salt lake	yellow	circular	entire	convex
58	BQSOILII M-83	HQ683724.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	yellow	circular	entire	convex
60	BQSOILII M-89(1)	JQ690689.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	hypersaline oil reservoir	cream	circular	entire	flat

Appendix F: Continuation

1	BQSOILII LB-2	EU912461.1	*Bacillus sp.*	Firmicutes	Bacilli	97%/93%	leaf	white	punctiform	entire	flat
2	BQSOILII LB-3(3)(2)	HQ259955.1	*Lysinibacillus fusiformis*	Firmicutes	Bacilli	100%/99%	intestine	cream	circular	erose	flat
4	BQSOILII LB-5(2)(1)	HQ610620.1	*Lysinibacillus fusiformis*	Firmicutes	Bacilli	100%/99%	vermicompost	white	circular	erose	flat
5	BQSOILII LB-6(2)	JQ579630.1	*Lysinibacillus fusiformis*	Firmicutes	Bacilli	100%/99%	peanut plant	cream	circular	curled	flat
6	BQSOILII LB-7	JX077088.1	*Bacillus anthracis*	Firmicutes	Bacilli	100%/100%	fresh leaf	white	filamentous	mentous/cur	flat
7	BQSOILII LB-8(1)	JN208085.1	*Bacillus safensis*	Firmicutes	Bacilli	100%/100%	marsh and two salterns	cream	circular	undulate	flat
8	BQSOILII LB-10(1)	JX077088.1	*Bacillus anthracis*	Firmicutes	Bacilli	100%/100%	fresh leaf	white	irregular	mentous/cur	convex
9	BQSOILII LB-10(2)	JX035965.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	diseased leaf	white	irregular	erose	convex
10	BQSOILII LB-12	JX077093.1	*Bacillus cereus*	Firmicutes	Bacilli	100%/100%	fresh leaf	white	circular	filamentous	flat
11	BQSOILII LB-15	JF820115.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil from Jianghan oil field	cream	circular	erose	flat
12	BQSOILII LB-17	JQ864365.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	Gut of Reticulitermes chinensis Snyder	yellow	rhizoid	lobate	flat
14	BQSOILII LB-20(2)	JF701969.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	brown	circular	undulate	convex
15	BQSOILII LB-22	JQ798393.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	sediment in sea cucumber	clear	circular	undulate	flat
16	BQSOILII LB-23(1)	AB533675.1	*Bacillus sp.*	Firmicutes	Bacilli	24%/90%	salt pan	white	circular	lobate	flat
17	BQSOILII LB-23(2)	JN700167.1	*Bacillus tequilensis*	Firmicutes	Bacilli	100%/100%	Root	cream	circular	entire	flat
18	BQSOILII LB-26	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	yellow	irregular	curled	crateriform
19	BQSOILII LB-27	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	yellow	circular	mentous/cur	flat
20	BQSOILII LB-28(2)	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	clear	circular	entous/und	flat
21	BQSOILII LB-29	JQ308558.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	geocarposphere soil	white	irregular	undulate	flat
22	BQSOILII LB-31(1)	JQ798393.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	sediment in sea	white	circular	entire	flat
23	BQSOILII LB-32(1)	JQ435677.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	wetland	cream	circular	undulate	flat
24	BQSOILII LB-32(2)	JQ435677.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	wetland	white	circular	entire	flat
25	BQSOILII LB-33	JQ798393.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	sediment in sea	clear	irregular	undulate	convex
26	BQSOILII LB-34	AB647202.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	palm oil contaminated soil	clear	circular	entire	flat
27	BQSOILII LB-35(1)	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	cream	circular	undulate	flat
28	BQSOILII LB-36	HQ259954.1	*Lysinibacillus fusiformis*	Firmicutes	Bacilli	100%/99%	intestine	white	circular	mentous/cur	flat
29	BQSOILII LB-38	GQ985395.2	*Paenibacillus sp.*	Firmicutes	Bacilli	100%/98%	soil	cream	circular	undulate	flat
30	BQSOILII LB-39	EU629346.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	alkaline digestive tract of Rhinoceros beetle	white	circular	undulate	flat
31	BQSOILII LB-44	HQ588864.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil sample from an industrial site	white	circular	entire	flat
32	BQSOILII LB-48	JQ435677.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	wetland	white	circular	entire	flat
1	BQSOILII Q-1	NR_044185.1	*Nocardioides terrigena*	Actinobacteria	Actinobacteria	100%/99%	soil	yellow	circular	entire	flat
3	BQSOILII Q-5	HQ398409.1	*Micromonospora sp.*	Actinobacteria	Actinobacteria	100%/100%	marine sediments	brown	circular	curled	convex
4	BQSOILII Q-7	EU741242.1	*Gordonia sp.*	Actinobacteria	Actinobacteria	100%/94%	beach sand	white	circular	entire	flat
6	BQSOILII Q-12	FR727718.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	forest soil	yellow	circular	entire	convex
7	BQSOILII Q-14	JF682781.1	*Streptomyces coeruleorubidus*	Actinobacteria	Actinobacteria	100%/100%	soil	white	circular	entire	flat
8	BQSOILII Q-19	JQ716226.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	soil	yellow	circular	entire	flat
9	BQSOILII Q-20	JF893647.1	*Uncultured Corynebacterium sp.*	Actinobacteria	Actinobacteria	21%/83%	bronchoalveolar lavage	cream	circular	undulate	flat
10	BQSOILII Q-21	JQ036309.1	*Nocardia sp.*	Actinobacteria	Actinobacteria	100%/100%	soil	clear	punctiform	lobate	flat
11	BQSOILII Q-22	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	cream	circular	curled	flat
12	BQSOILII Q-23	JN942128.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	crude oil-contaminated surface water and sponges	yellow	circular	curled	umbonate
13	BQSOILII Q-25	JN969032.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	soil of chemically polluted	yellow	circular	filamentous	convex
14	BQSOILII Q-26	GU244504.1	*Novosphingobium sp.*	Proteobacteria	α	100%/99%	surface seawater	yellow	circular	entire	flat
15	BQSOILII Q-28	JN578481.1	*Bacillus sp.*	Firmicutes	Bacilli	99%/95%	soil	cream	circular	undulate	flat
16	BQSOILII Q-29	EU741199.1	*Streptomyces chrestomyceticus*	Actinobacteria	Actinobacteria	100%/100%	soil at hightide line	gray/brown	circular	curled	umbonate
17	BQSOILII Q-30	JQ782962.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	marine sediment	white/yellow	circular	filamentous	convex
18	BQSOILII Q-31	GU321991.1	*Paenibacillus stellifer*	Firmicutes	Bacilli	100%/99%	plant rhizospheres	white	circular	undulate	flat
20	BQSOILII Q-34	JQ688016.1	*Micromonospora sp.*	Actinobacteria	Actinobacteria	100%/100%	Tubastraea coccinea (coral)	brown	circular	entire	flat
21	BQSOILII Q-35	JQ518348.1	*Mycobacterium sp.*	Actinobacteria	Actinobacteria	100%/99%	soil	cream	irregular	lobate	flat

Appendix F: Continuation

1	BQSOILII S-2	GU217694.1	*Kocuria sp.*	Actinobacteria	Actinobacteria	100%/99%	mud volcano 1281 meters above sea	white	circular	entire	flat
2	BQSOILII S-5	JQ040006.1	*Rhodococcus aetherivorans*	Actinobacteria	Actinobacteria	100%/100%	soil	orange	circular	entire	raised
4	BQSOILII S-8	JQ716226.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	soil	yellow	circular	entire	convex
5	BQSOILII S-9	HM480336.1	*Bacillus megaterium*	Firmicutes	Bacilli	85%/82%	paper mill	cream	circular	undulate	flat
6	BQSOILII S-10	JX035965.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	diseased leaf	brown	circular	entire	flat
7	BQSOILII S-11	JQ716226.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	soil	yellow	circular	entire	convex
8	BQSOILII S-13	EU332823.1	*Paenibacillus sp.*	Firmicutes	Bacilli	100%/99%	soil of ginseng field	cream	circular	undulate	flat
9	BQSOILII S-15	JF273850.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/99%	soil	brown	circular	entire	flat
10	BQSOILII S-16	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	cream	circular	erose	flat
11	BQSOILII S-17	EU982515.1	*Paenibacillus polymyxa*	Firmicutes	Bacilli	100%/99%	retting sewage	white	circular	undulate	flat
12	BQSOILII S-20	JN585701.1	*Arthrobacter sp.*	Actinobacteria	Actinobacteria	100%/99%	rhizospheric soil of Arachis hypogaea	white	irregular	entire	flat
13	BQSOILII S-21	AB495170.1	*Arthrobacter sp.*	Actinobacteria	Actinobacteria	100%/99%	hydroponic cultures of moss Racomitrium japonicum	yellow	circular	entire	flat
15	BQSOILII S-23	JN969032.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	soil of chemically polluted	white	filamentous	filamentous	convex
16	BQSOILII S-24(1)(1)	JF701969.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	cream	circular	undulate	flat
17	BQSOILII S-24(1)(2)	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	yellow	circular	entire	flat
18	BQSOILII S-24(2)	JF701969.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	clear	irregular	entous/und	flat
19	BQSOILII S-26	DQ448706.1	*Microbacterium sp*	Actinobacteria	Actinobacteria	100%/99%	marine sediment	yellow	circular	entire	flat
20	BQSOILII S-27	HE586887.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	marine sediment	white	punctiform	lobate	flat
21	BQSOILII S-28	GQ918257.1	*Brevibacillus borstelensis*	Firmicutes	Bacilli	100%/100%	wastewater sludge	brown	punctiform	entire	flat
22	BQSOILII S-29	JN208097.1	*Bacillus niabensis*	Firmicutes	Bacilli	100%/98%	marsh and two salterns	white	circular	erose	flat
23	BQSOILII S-30	JN210907.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	brown	circular	undulate	convex
24	BQSOILII S-31	JX077093.1	*Bacillus cereus*	Firmicutes	Bacilli	100%/100%	fresh leaf	white	circular	entire	flat
25	BQSOILII S-34	JN592473.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	non-flooded rice field	cream	irregular	undulate	flat
26	BQSOILII S-35	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	99%/100%	deep-sea surface sediment	white	circular	entire	flat
27	BQSOILII S-39	JF917312.1	*Microbacterium sp.*	Actinobacteria	Actinobacteria	100%/100%	seagrass	yellow	circular	entire	raised
28	BQSOILII S-40(1)	EU497638.1	*Paenibacillus sp.*	Firmicutes	Bacilli	100%/98%	soil	white	circular	entire	flat
29	BQSOILII S-41(1)(1)	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	clear	irregular	undulate	flat
30	BQSOILII S-42	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	99%/100%	deep-sea surface sediment	cream	circular	entire	flat
31	BQSOILII S-43	GU350488.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	soil	white/yellow	irregular	lobate	umbonate

Appendix G: Hindgut microflora from *Uca rapax* (Wet season).

DNA/PCR Codes	Plates Codes	Accesion Number	Identification	Phylum	Class	Query Coverage / Max Identity	Source	Color	Shape	Border	Elevation
1	BQ IIM-1(1)(1)	JN195795.1	*Exiguobacterium profundum*	Firmicutes	Bacilli	99% / 99%	cement-sand	yellow	circular	undulate	flat
2	BQ IIM-3(1)(1)	HQ844969.1	*Bacillus pumilus*	Firmicutes	Bacilli	99% / 99%	sludge	white	irregular	curled	umbonate
3	BQ IIM-3(1)(2)	EU878269.1	*Bacillus sp.*	Firmicutes	Bacilli	98% / 99%	soil	clear	irregular	lobate	flat
4	BQ IIM-4(2)(2)(2)	JF909359.1	*Cellulosimicrobium sp.*	Actinobacteria	Actinobacteridae	98% / 99%	soil	yellow	punctiform	filamentous	flat
5	BQ IIM-4(3)(1)	JQ435677.1	*Bacillus pumilus*	Firmicutes	Bacilli	97% / 99%	wetland	white	irregular	undulate	flat
6	BQ IIM-4(3)(2)	FN357284.1	*Rhodococcus sp.*	Actinobacteria	Actinobacteridae	100% / 99%	contaminated soil	orange	rhizoid	lobate	convex
7	BQ IIM-5(1)(1)	GQ169785.1	*Bacillus pumilus*	Firmicutes	Bacilli	98% / 97%	stem	clear	irregular	undulate	curled
8	BQ IIM-5(1)(3)(1)	FJ485830.1	*Bacillus pumilus*	Firmicutes	Bacilli	98% / 99%	soil	yellow	circular	entire	flat
9	BQ IIM- 5(3)	HQ285772.1	*Paenibacillus xylanilyticus*	Firmicutes	Bacilli	93% / 99%	Antarctic ice	white	irregular	lobate	flat
10	BQ IIM-7(1)(1)	EU874877.1	*Bacillus cereus*	Firmicutes	Bacilli	90% / 78%	East China Sea	gray	rhizoid	filamentous	raised
11	BQ IIM-7(3)(1)	JN593079.1	*Bacillus sp.*	Firmicutes	Bacilli	98% / 99%	soil	white	irregular	filamentous	flat
12	BQ IIM-8(2)(2)	HQ143613.1	*Bacillus sp.*	Firmicutes	Bacilli	82% / 97%	soil	clear	circular	entire	flat
13	BQ IIM-8(3)	AY484507.1	*Bacillus fusiformis*	Firmicutes	Bacilli	79% / 94%	rhizosphere	white	irregular	undulate	convex
14	BQ IIM-9(3)(1)	HQ202812.1	*Microbacterium oxydans*	Actinobacteria	Actinobacteridae	75% / 90%	soil	orange	irregular	undulate	flat
15	BQ IIM-9(3)(2)	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	93% / 100%	crude oil-contaminated surface water	yellow	circular	entire	flat
16	BQ IIM-10(3)	GQ462533.1	*Bacillus cereus*	Firmicutes	Bacilli	99% / 99%	wastewater of silk industry	white	irregular	filamentous	flat
17	BQ IIM-11(2)	JN256920.1	*Bacillus sp.*	Firmicutes	Bacilli	99% / 86%	flooded rice soil	clear	circular	undulate	flat
18	BQ IIM-12(1)(1)(2)	EU834272.1	*Bacillus pumilus*	Firmicutes	Bacilli	98% / 86%	soil	yellow	punctiform	erose	convex
19	BQ IIM-13(2)	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	93% / 100%	crude oil-contaminated surface water	yellow	circular	entire	convex
20	BQ IIM-13(3)	EU603457.1	*Pseudomonas pachastrellae*	Proteobacteria	γ	98% / 92%	ocean sediment	white	circular	undulate	convex
21	BQ IIM-13(4)(1)	JN999846.1	*Bacillus marisflavi*	Firmicutes	Bacilli	94% / 99%	agricultural soil	yellow	circular	entire	flat
22	BQ IIM-14(1)	HM104646.1	*Bacillus cereus*	Firmicutes	Bacilli	54% / 88%	soil	white	filamentous	filamentous	flat
23	BQ IIM-15(2)(3)	FJ390482.1	*Bacillus sp.*	Firmicutes	Bacilli	55% / 94%	alpine grassland	white	circular	entire	flat
24	BQ IIM-17(1)(1)	EU584536.1	*Bacillus sp.*	Firmicutes	Bacilli	56% / 88%	rolling wastewater	cream	irregular	undulate	flat
25	BQ IIM-17(2)	GU391510.1	*Bacillus sp.*	Firmicutes	Bacilli	95% / 96%	roots	white		filamentous	
26	BQ IIM-20(3)	EU594558.1	*Bacillus pumilus*	Firmicutes	Bacilli	99% / 99%	root and leaves	white	irregular	lobate	flat
27	BQ IIM-29(1)(1)	HQ219921.1	*Bacillus sp.*	Firmicutes	Bacilli	99% / 99%	roots	yellow	circular	entire	convex
28	BQ IIM-29(2)(1)	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	97% / 99%	seagrass	yellow	circular	entire	convex
1	BQ IILB-1(1)	HQ219921.1	*Bacillus sp.*	Firmicutes	Bacilli	99% / 99%	roots	white	filamentous	filamentous	flat
3	BQ IILB-13(2)	JN593079.1	*Bacillus sp.*	Firmicutes	Bacilli	97% / 99%	soil	white	irregular	curled	convex
4	BQ IILB-14(2)	EU584531.1	*Bacillus sp.*	Firmicutes	Bacilli	42% / 96%	rolling wastewater	white	irregular	curled	convex
5	BQ IILB-15(1)(1)	HQ143609.1	*Bacillus pumilus*	Firmicutes	Bacilli	98% / 99%	rhizosphere	white	irregular	lobate	flat
6	BQ IILB-15(2)	HQ259954.1	*Lysinibacillus fusiformis*	Firmicutes	Bacilli	34% / 92%	intestine	clear	rhizoid	filamentous	convex
7	BQ IILB-18(1)	JN999848.1	*Bacillus anthracis*	Firmicutes	Bacilli	94% / 99%	agricultural soil	white	irregular	filamentous/curled	convex
8	BQ IILB-22(1)	JF784645.1	*Bacillus cereus*	Firmicutes	Bacilli	40% / 91%	rhizosphere soil	white	irregular	erose	flat
9	BQ IILB-21(2)	HQ143640.1	*Geobacillus stearothermophilus*	Firmicutes	Bacilli	96% / 80%	soil	white	circular	entire	flat
10	BQ IILB-23(1)	GU479395.1	*Bacillus sp.*	Firmicutes	Bacilli	98% / 99%	pesticide contaminated soil	yellow	circular	entire	flat
11	BQ IILB-25(1)	JF343154.1	*Bacillus megaterium*	Firmicutes	Bacilli	96% / 79%	lake sediments	cream	circular	filamentous	flat
12	BQ IILB-27(2)(1)(1)(1)(1)	JQ292902.1	*Lysinibacillus sphaericus*	Firmicutes	Bacilli	95% / 98%	plants	cream	circular	filamentous	flat
13	BQ IILB-27(3)(1)	FJ233851.1	*Lysinibacillus sp.*	Firmicutes	Bacilli	99% / 99%	Kizhanelli root	white	irregular	entire	flat
14	BQ IILB-28	JN700163.1	*Lysinibacillus sphaericus*	Firmicutes	Bacilli	78% / 82%	Plant	yellow	irregular	lobate	flat
15	BQ IILB-30	JQ650110.1	*Vibrio fluvialis*	Proteobacteria	γ	96% / 99%	mussel	white	circular	entire	flat
16	BQ IILB-31(2)	HM769816.1	*Bacillus cereus*	Firmicutes	Bacilli	84% / 87%	N/A	clear	irregular	undulate	flat
17	BQ IILB-32	JQ650110.1	*Vibrio fluvialis*	Proteobacteria	γ	98% / 97%	mussel	yellow	circular	entire	flat
18	BQ IILB-33(1)	JQ650110.1	*Vibrio fluvialis*	Proteobacteria	γ	70% / 96%	mussel	white	circular	entire	flat
19	BQ IILB-36(1)(1)(1)	GU329917.1	*Bacillus cereus*	Firmicutes	Bacilli	96% / 99%	sewage treatment plant	clear	circular	lobate	umbonate
20	BQ IILB-36(1)(2)(1)	JF460740.1	*Bacillus cereus*	Firmicutes	Bacilli	98% / 99%	tobacco	clear	irregular	erose	flat

Appendix G: Continuation

1	BQ IIQ-1(1)	JN791375.1	*Isoptericola sp.*	Actinobacteria	Actinobacteridae	95% / 100%	marine sediment	white	circular	filamentous	convex
2	BQ IIQ-1(4)(2)	GQ284526.1	*Microbacterium sp*	Actinobacteria	Actinobacteridae	57% / 97%	mangrove sediment	orange	circular	entire	umbonate
3	BQ IIQ-(2)(1)	JQ518340.1	*Streptomyces sp.*	Actinobacteria	Actinobacteridae	93% ;/ 100%	surface of soil	gray	filamentous	filamentous	pulvinate
4	BQ IIQ-3	HQ585880.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	65% / 78%	roots of sugarcane	white/yellow	filamentous	filamentous	raised
5	BQ IIQ-4	JN862830.1	*Streptomyces djakartensis*	Actinobacteria	Actinobacteridae	97% / 100%	soils	white/yellow	circular	filamentous	flat
6	BQ IIQ-5(2)	GU991528.1	*Gordonia sp.*	Actinobacteria	Actinobacteridae	97% / 99%	soil	orange	punctiform	entire	convex
7	BQ IIQ-6(3)	AJ399493.1	*Streptomyces neyagawaensis*	Actinobacteria	Actinobacteridae	97% / 99%	soil	white	circular	filamentous	convex
8	BQ IIQ-6(4)(2)	GQ478415.1	*Cellulosimicrobium sp.*	Actinobacteria	Actinobacteridae	98% / 99%	Lower Digestive Tract	yellow	circular	filamentous	umbonate
9	BQ IIQ-6(4)(3)	AB506120.1	*Rhodococcus pyridinivorans*	Actinobacteria	Actinobacteridae	97% / 100%	agriculture soil	pink	punctiform	entire	convex
10	BQ IIQ-7(1)	DQ792510.1	*Streptomyces sp.*	Actinobacteria	Actinobacteridae	97% / 99%	rhizosphere soil	white	filamentous	filamentous	flat
11	BQ IIQ-8(1)	AM990831.1	*Streptomyces sp.*	Actinobacteria	Actinobacteridae	97% / 99%	sea water	brown	circular	entire	convex
12	BQ IIQ-8(2)	FJ851360.1	*Gordonia sp.*	Actinobacteria	Actinobacteridae	97% / 100%	soil	orange	irregular	undulate	convex
13	BQ IIQ-9(1)	FJ626635.1	*Streptomyces sp.*	Actinobacteria	Actinobacteridae	98% / 99%	N/A	white/yellow	circular	filamentous/curled	convex
14	BQ IIQ-9(2)	EU741088.1	*Paracoccus sp.*	Proteobacteria	α	98% / 99%	sand	white	circular	entire	flat
15	BQ IIQ-10(2)(1)	EU834263.1	*Microbacterium foliorum*	Actinobacteria	Actinobacteridae	99% / 99%	soil	yellow	irregular	entire	flat
16	BQ IIQ-10(2)(2)	EU741088.1	*Paracoccus sp.*	Proteobacteria	α	98% / 99%	sand	clear	circular	entire	convex
17	BQ IIQ-12(2)	AJ250800.1	*Bosea thiooxidans*	Proteobacteria	α	98% / 99%	rhizoplane	white	circular	entire	flat
18	BQ IIQ-13(1)	FJ834432.1	*Bacillus cereus*	Firmicutes	Bacilli	83% / 94%	ship scrapping	white	filamentous	filamentous	umbonate
19	BQ IIQ-14(1)	JN859008.1	*Streptomyces sp.*	Actinobacteria	Actinobacteridae	95% / 99%	saltpan	white	circular	entire	flat
21	BQ IIQ-15(2)	EF157823.1	*Pseudomonas aeruginosa*	Proteobacteria	γ	37% / 98%	rhizosphere	white	circular	entire	flat
22	BQ IIQ-16(1)(1)	FJ001754.1	*Streptomyces sp.*	Actinobacteria	Actinobacteridae	37% / 95%	woody tree roots	white	circular	entire	flat
23	BQ IIQ-16(3)	JN791308.1	*Echinicola sp.*	Bacteroidetes	Cytophagia	48% / 96%	marine sediment	pink	irregular	undulate	flat
24	BQ IIQ-16(4)	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	98% / 99%	crude oil-contaminated surface water	yellow	circular	entire	flat
25	BQ IIQ-17(1)(1)(1)	JN585718.1	*Achromobacter xylosoxidans*	Proteobacteria	β	24% / 91%	rhizospheric soil	white	circular	curled	convex
26	BQ IIQ-17(1)(1)(2)	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 99%	crude oil-contaminated surface water	yellow	irregular	entire	flat
27	BQ IIQ-17(1)(2)	JN585717.1	*Achromobacter xylosoxidans*	Proteobacteria	β	83% / 96%	rhizospheric soil	white	circular	entire	flat
28	BQ IIQ-17)3)(2)	GQ867055.1	*Streptomyces sp.*	Actinobacteria	Actinobacteridae	90% / 78%	soil	white	rhizoid	filamentous	flat
29	BQ IIQ-18(2)(2)	JF701945.1	*Bacillus sp.*	Firmicutes	Bacilli	98% / 99%	soil	white	filamentous	filamentous	raised
30	BQ IIQ-18(3)	HQ202812.1	*Microbacterium oxydans*	Actinobacteria	Actinobacteridae	61% / 82%	soil	yellow	circular	entire	flat
31	BQ IIQ-19(1)	JQ291586.1	*Cellulosimicrobium funkei*	Actinobacteria	Actinobacteridae	52% / 97%	larvae gut	white	circular	filamentous	crateriform

Appendix G: Continuation

1	BQ IIS-1(3)	HQ398389.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	98% / 83%	marine sponge	yellow	circular	filamentous	umbonate
2	BQ IIS-1(4)	JF909359.1	*Cellulosimicrobium sp.*	Actinobacteria	Actinobacteridae	98% / 99%	soil	yellow	circular	filamentous	raised
3	BQ IIS-3(1)	EU037292.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 91%	chromium contaminated soil	orange	irregular	undulate	pulvinate
4	BQ IIS-3(5)(1)	EU124555.1	*Bacillus megaterium*	Firmicutes	Bacilli	100% / 99%	contaminated soil from metallurgic	clear	circular	entire	flat
5	BQ IIS-3(5)(2)	EU124555.1	*Bacillus megaterium*	Firmicutes	Bacilli	99% / 99%	contaminated soil from metallurgic	clear	circular	curled	convex
6	BQ IIS-4(1)	JF909359.1	*Cellulosimicrobium sp.*	Actinobacteria	Actinobacteridae	97% / 99%	soil	yellow	circular	filamentous	convex
7	BQ IIS-4(3)	GU321990.1	*Paenibacillus stellifer*	Firmicutes	Bacilli	98% / 99%	plant rhizospheres	white	circular	erose	flat
8	BQ IIS-5(1)	EU741088.1	*Paracoccus sp.*	Proteobacteria	α	98% / 98%	sand	cream	circular	entire	flat
10	BQ IIS-6(3)(1)	HM222667.1	*Cellulomonas sp.*	Actinobacteria	Actinobacteridae	93% / 100%	deep-sea sediment	yellow	circular	entire	flat
11	BQ IIS-7(3)	EU834263.1	*Microbacterium foliorum*	Actinobacteria	Actinobacteridae	99% / 99%	soil	yellow	circular	entire	flat
12	BQ IIS-7(5)(1)	HM222667.1	*Cellulomonas sp.*	Actinobacteria	Actinobacteridae	97% / 93%	deep-sea sediment	yellow	punctiform	entire	flat
13	BQ IIS-7(5)(4)	JQ039981.1	*Brevibacillus invocatus*	Firmicutes	Bacilli	96% / 92%	root	white	punctiform	curled	flat
14	BQ IIS-8	JF917312.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 99%	seagrass	yellow	circular	entire	flat
15	BQ IIS-10(1)	JF274934.1	*Paenibacillus sp.*	Firmicutes	Bacilli	66% / 82%	olive-mill wastewater	yellow	circular	entire	flat
16	BQ IIS-10(3)	GQ478418.1	*Cellulosimicrobium sp.*	Actinobacteria	Actinobacteridae	97% / 100%	Lower Digestive Tract	yellow	punctiform	filamentous	flat
17	BQ IIS-14(1)	NR_028823.1	*Gordonia sinesedis*	Actinobacteria	Actinobacteridae	97% / 99%	soil	pink	circular	entire	raised
18	BQ IIS-15(2)(1)	JN627170.1	*Gordonia bronchialis*	Actinobacteria	Actinobacteridae	97% / 97%	soft coral	orange	circular	entire	flat
19	BQ IIS-16(1)(1)	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	98% / 88%	crude oil-contaminated surface water	yellow	circular	entire	flat
20	BQ IIS-16(3)	HM453885.1	*Brevibacillus sp*	Firmicutes	Bacilli	94% / 97%	rhizosphere soil	white	circular	filamentous	flat
21	BQ IIS-17(1)	HM453885.1	*Brevibacillus sp*	Firmicutes	Bacilli	78% / 96%	rhizosphere soil	white	circular	filamentous	flat
22	BQ IIS-20(30(1)	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	93% / 99%	crude oil-contaminated surface water	yellow	punctiform	entire	flat
23	BQ IIS-21(1)	JN644529.1	*Microbacterium oxydans*	Actinobacteria	Actinobacteridae	98% / 99%	midgut	yellow	circular	entire	flat
24	BQ IIS-22(1)	HM453885.1	*Brevibacillus sp*	Firmicutes	Bacilli	82% / 96%	rhizosphere soil	white	irregular	erose	flat
25	BQ IIS-23(1)(1)	HQ843844.1	*Brevibacillus parabrevis*	Firmicutes	Bacilli	89% / 88%	rhizosphere	white	irregular	erose	flat
26	BQ IIS-24	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 100%	crude oil-contaminated surface water	yellow	circular	undulate	flat
27	BQ IIS-25(1)	JN644517.1	*Microbacterium oxydans*	Actinobacteria	Actinobacteridae	98% / 99%	midgut	yellow	circular	lobate	flat
28	BQ IIS-25(3)	JN862838.1	*Streptomyces atratus*	Actinobacteria	Actinobacteridae	97% / 99%	soil	purple	filamentous	filamentous	convex
29	BQ IIS-26(1)(2)	JN999878.1	*Brevibacillus choshinensis*	Firmicutes	Bacilli	96% / 91%	soil	white	circular	entire	flat
30	BQ IIS-27(2)	JN942151.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	98% / 100%	crude oil-contaminated surface water	yellow	circular	entire	flat
31	BQ IIS-28(3)	AJ971868.1	*Paenibacillus sp*	Firmicutes	Bacilli	36% / 94%	gut of bumble bee	white	irregular	undulate	flat
32	BQ IIS-29(1)	GU056313.1	*Pseudomonas sp.*	Proteobacteria	γ	98% / 99%	River Ganges	yellow	irregular	lobate	flat
33	BQ IIS-30(1)	EU906929.1	*Streptomyces sp.*	Actinobacteria	Actinobacteridae	97% / 99%	sediment of tidal flat	yellow	circular	entire	flat
35	BQ IIS-32(1)	HM222659.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	91% / 84%	deep-sea sediment	clear	circular	entire	flat
36	BQ IIS-32(2)	AB552874.1	*Microbacterium sp.*	Actinobacteria	Actinobacteridae	97% / 86%	Algal-bacterial consortia	yellow	circular	curled	flat
37	BQ IIS034(3)	HQ831417.1	*Streptomyces atroolivaceus*	Actinobacteria	Actinobacteridae	97% / 100%	tobacco	white	filamentous	filamentous	pulvinate
38	BQ IIS-37	AB453290.1	*Enterobacter sp.*	Proteobacteria	γ	99% / 99%	soil	clear	circular	entire	flat
39	BQ IIS-38(1)	DQ916277.2	*Pseudomonas lindanilytica*	Proteobacteria	γ	99% / 98%	pesticide contaminated soil	white	filamentous	filamentous	flat
40	BQ IIS-39(1)	HM584796.1	*Klebsiella sp.*	Proteobacteria	γ	98% / 99%	intestines	clear	circular	entire	flat
41	BQ IIS-41	HM584796.1	*Klebsiella sp.*	Proteobacteria	γ	99% / 98%	intestines	white	rhizoid	filamentous	flat
42	BQ IIS-42(1)	HQ224640.1	*Pseudomonas sp.*	Proteobacteria	γ	96% / 85%	N/A	white	irregular	lobate	flat
43	BQ IIS-45(1)	JF783992.1	*Serratia sp.*	Proteobacteria	γ	75% / 89%	tobacco	white	irregular	undulate	flat
45	BQ IIS-47(1)	HM582426.1	*Pseudomonas aeruginosa*	Proteobacteria	γ	61% / 97%	tuberose rhizosphere	yellow	circular	entire	flat
46	BQ IIS-47(2)	EU584546.1	*Bacillus sp.*	Firmicutes	Bacilli	33% / 99%	wastewater	white	irregular	filamentous	flat

Appendix H: Hindgut microflora from *Uca rapax* (Dry season).

DNA/PCR Codes	Plates Codes	Accesion Number	Identification	Phylum	Class	Query Coverage / Max Identity	Source	Color	Shape	Border	Elevation
1	BQIII M-1	JX077095.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	tobacco	yellow	punctiform	erose	convex
3	BQIII M-5	JF719277.1	*Ruegeria sp.*	Proteobacteria	α	100%/98%	ocean water	brown	circular	entire	flat
4	BQIII M-7	GU397426.2	*Halomonas smyrnensis*	Proteobacteria	γ	100%/99%	salt production pond	white	circular	entire	flat
5	BQIII M-9	FR822983.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/98%	soil	cream	circular	entire	flat
6	BQIII M-12	JN791286.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	marine sediment	cream	circular	entire	flat
7	BQIII M-13	JQ799125.1	*Vibrio proteolyticus*	Proteobacteria	γ	100%/99%	tropical marine sediment from jetty	cream	circular	entire	flat
8	BQIII M-14	JX035965.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	diseased leaf	cream	circular	entire	convex
9	BQIII M-16	NR_044243.1	*Microbulbifer celer*	Proteobacteria	γ	100%/99%	marine solar saltern	white	circular	undulate	convex
10	BQIII M-18	JQ030912.1	*Bacillus aquimaris*	Firmicutes	Bacilli	100%/100%	sediment	white	punctiform	entire	flat
11	BQIII M-19	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	99%/99%	seagrass	brown	irregular	filamentous	flat
12	BQIII M-20	NR_044243.1	*Microbulbifer celer*	Proteobacteria	γ	100%/99%	marine solar saltern	cream	circular	entire	convex
13	BQIII M-21	NR_044243.1	*Microbulbifer celer*	Proteobacteria	γ	100%/99%	marine solar saltern	yellow	circular	entire	flat
14	BQIII M-22	JF775424.1	*Bacillus aquimaris*	Firmicutes	Bacilli	100%/100%	Plant	yellow	irregular	undulate	flat
15	BQIII M-24	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	white	irregular	erose	convex
17	BQIII M-26	AB617569.1	*Vibrio harveyi*	Proteobacteria	γ	100%/100%	solar salt from saltern	cream	circular	entire	convex
18	BQIII M-27	JN800356.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	polluted river sediment	yellow	circular	erose	convex
20	BQIII M-33	AB617569.1	*Vibrio harveyi*	Proteobacteria	γ	100%/99%	solar salt from saltern	cream	circular	entire	flat
21	BQIII M-34	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	white	circular	entire	convex
22	BQIII M-35	FJ527419.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	forest soil sample	clear	circular	entire	flat
23	BQIII M-41	JN999846.1	*Bacillus marisflavi*	Firmicutes	Bacilli	100%/100%	agricultural soil	cream	circular	entire	flat
24	BQIII M-42a	JF701954.1	*Bacillus sp.*	Firmicutes	Bacilli	99%/99%	soil	white	circular	entire	flat
26	BQIII M-43(2)	FJ809934.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	roots	white	circular	entire	flat
27	BQIII M-44	GQ903459.1	*Halobacillus litoralis*	Firmicutes	Bacilli	100%/100%	soil of salt lake	yellow	circular	entire	flat
28	BQIII M-45	GU085229.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil near an oil refiner	white	circular	undulate	flat
29	BQIII M-47	JQ904733.1	*Vibrio parahaemolyticus*	Proteobacteria	γ	100%/100%	surface sediment	yellow	circular	entire	flat
30	BQIII M-48	GQ205448.1	*Vibrio parahaemolyticus*	Proteobacteria	γ	100%/99%	Penaeus vannamei (Whiteleg shrimp)	yellow	circular	erose	flat
31	BQIII M-49	AB617551.1	*Bacillus marisflavi*	Firmicutes	Bacilli	100%/100%	solar salt from saltern	yellow	circular	curled	convex
32	BQIII M-50	FJ444948.1	*Halobacillus trueperi*	Firmicutes	Bacilli	100%/99%	Saltern	yellow	circular	entire	flat
33	BQIII M-51	JF820115.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil from Jianghan oil field	yellow	circular	entire	flat
34	BQIII M-52	AY967717.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	coastal sea water	pink	circular	entire	convex
35	BQIII M-54	FJ607059.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	sediment	yellow	circular	entire	flat
36	BQIII M-55	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	yellow	circular	entire	flat
37	BQIII M-56(2)	HQ161732.1	*Vibrio harveyi*	Proteobacteria	γ	100%/99%	Dysidea granulosa	yellow	circular	entire	flat
38	BQIII M-59	GU223379.1	*Marinobacter sp.*	Proteobacteria	γ	100%/99%	ocean water	yellow	circular	entire	convex
39	BQIII M-63	FJ527419.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	soil	yellow	circular	entire	flat
40	BQIII M-64	FJ527419.1	*Microbulbifer sp.*	Proteobacteria	γ	100%/99%	soil	yellow	irregular	undulate	flat
41	BQIII M-65	HQ202857.1	*Bacillus selenatarsenatis*	Firmicutes	Bacilli	100%/99%	soil	clear	irregular	undulate	flat
42	BQIII M-66							yellow	circular	entire	flat
43	BQIII M-69	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	white	circular	undulate	crateriform
44	BQIII M-73	NR_044243.1	*Microbulbifer celer*	Proteobacteria	γ	100%/99%	marine solar saltern	brown	irregular	entire	flat
45	BQIII M-75	JN942138.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	crude oil-contaminated surface water and sponges	yellow	irregular	erose	flat
46	BQIII M-76(1)	JN999846.1	*Bacillus marisflavi*	Firmicutes	Bacilli	99%/100%	agricultural soil	yellow	circular	entire	flat
47	BQIII M-76(2)	AB539975.1	*Micrococcus luteus*	Actinobacteria	Actinobacteria	100%/100%	groundwater	yellow	circular	entire	pulvinate
48	BQIII M-77	HQ683725.1	*Halobacillus sp.*	Firmicutes	Bacilli	100%/100%	salt lake	yellow	circular	entire	flat
49	BQIII M-78	JN942138.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	crude oil-contaminated surface water and sponges	clear	circular	entire	flat
2	BQIII LB-1(2)	NR_044538.1	*Bacillus korlensis*	Firmicutes	Bacilli	100%/99%	desert soil sample	yellow	irregular	undulate	flat
3	BQIII LB-3	JF775424.1	*Bacillus aquimaris*	Firmicutes	Bacilli	100%/99%	endophytic plant	yellow	circular	curled	flat
4	BQIII LB-4	AB647202.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	palm oil contaminated soil	clear	punctiform	entire	flat
5	BQIII LB-5(2)	JN256920.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	flooded rice soil	clear	circular	entire	flat
6	BQIII LB-8	JF820115.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	soil from Jianghan oil field	white	irregular	erose	raised
7	BQIII LB-10	AB647202.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	palm oil contaminated soil	clear	irregular	undulate	raised
8	BQIII LB-13	AB647202.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	palm oil contaminated soil	clear	irregular	lobate	flat
9	BQIII LB-15	AB647202.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	palm oil contaminated soil	white	irregular	lobate	flat
10	BQIII LB-17	JN128250.1	*Bacillus aryabhattai*	Firmicutes	Bacilli	100%/100%	marine sponge	yellow	irregular	lobate	flat
11	BQIII LB-18	EU221375.1	*Bacillus niacini*	Firmicutes	Bacilli	100%/100%	wheat rhizosphere	clear	circular	curled	flat
12	BQIII LB-19	JN700160.1	*Bacillus cereus*	Firmicutes	Bacilli	100%/99%	plant	orange	punctiform	entire	flat
13	BQIII LB-21	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	white	irregular	undulate	flat
15	BQIII LB-26	FJ784129.1	*Bacillus niabensis*	Firmicutes	Bacilli	100%/99%	Phyllostachys pubescens	clear	circular	entire	flat
16	BQIII LB-27	JN208097.1	*Bacillus niabensis*	Firmicutes	Bacilli	100%/99%	marsh	white	irregular	erose	flat
17	BQIII LB-29	JQ689191.1	*Lysinibacillus sphaericus*	Firmicutes	Bacilli	100%/100%	mineral water	yellow	circular	entire	pulvinate
18	BQIII LB-31	EU332823.1	*Paenibacillus sp.*	Firmicutes	Bacilli	100%/99%	soil of ginseng field	clear	circular	entire	flat
19	BQIII LB-32	FJ481961.1	*Bacillus pumilus*	Firmicutes	Bacilli	100%/100%	Asobara tabida	white	irregular	erose	flat
20	BQIII LB-35	JF346660.1	*Brachybacterium sp.*	Actinobacteria	Actinobacteria	100%/99%	coastal sea water	yellow	punctiform	entire	flat

Appendix H: Continuation

1	BQIII Q-1	JN180219.1	*Streptomyces libani*	Actinobacteria	Actinobacteria	100%/99%	soil	white	circular	filamentous	convex
2	BQIII Q-2	EU741242.1	*Gordonia sp.*	Actinobacteria	Actinobacteria	100%/96%	beach sand	orange	circular	entire	flat
3	BQIII Q-3	JN566189.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	99%/100%	soil	white	irregular	filamentous	flat
4	BQIII Q-6(1)	JN578481.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	yellow	circular	entire	convex
5	BQIII Q-7	FN422001.1	*Paenibacillus illinoisensis*	Firmicutes	Bacilli	99%/99%	garden soil	orange	circular	entire	flat
6	BQIII Q-8	JX051282.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/99%	coastal sediment	white	filamentous	filamentous	convex
8	BQIII Q-11	AB506120.1	*Rhodococcus pyridinivorans*	Actinobacteria	Actinobacteria	100%/99%	agriculture soil	pink	circular	lobate	umbonate
9	BQIII Q-12	DQ275185.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	granite	white	circular	erose	flat
10	BQIII Q-13(1)	DQ374636.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	n/a	clear	punctiform	filamentous	flat
11	BQIII Q-13(2)	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	99%/99%	seagrass	yellow	circular	entire	convex
12	BQIII Q-15	AB712352.1	*Streptomyces somaliensis*	Actinobacteria	Actinobacteria	100%/100%	deep-sea surface sediment	gray	circular	mentous/cur	convex
13	BQIII Q-16	JX051282.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	coastal sediment	yellow	irregular	filamentous	flat
14	BQIII Q-17	JN936847.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	chemical pollution environment	yellow	circular	filamentous	crateriform
16	BQIII Q-21	JF701918.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	earthworm's gut	gray	irregular	filamentous	pulvinate
17	BQIII Q-22	JN791314.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	marine sediment	yellow	circular	entire	convex
20	BQIII Q-29	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	99%/99%	seagrass	yellow	circular	entire	umbonate
21	BQIII Q-31	JQ031555.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/99%	sea sediment	white	filamentous	curled	umbonate
23	BQIII Q-34	DQ448699.1	*Gordonia sp.*	Actinobacteria	Actinobacteria	100%/96%	marine sediment	pink	circular	entire	convex
24	BQIII Q-37(2)	JN208059.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	marsh	brown	irregular	curled	convex
1	BQIII S-1(1)	JQ936679.1	*Bacillus flexus*	Firmicutes	Bacilli	100%/100%	leaf	yellow	irregular	lobate	convex
2	BQIII S-2	JQ659928.1	*Bacillus aryabhattai*	Firmicutes	Bacilli	100%/99%	plant tissue	white	irregular	undulate	flat
3	BQIII S-3	AB697153.1	*Bacillus megaterium*	Firmicutes	Bacilli	99%/100%	Apple leaf	gray	irregular	entire	flat
4	BQIII S-4	JQ658424.1	*Gordonia sp.*	Actinobacteria	Actinobacteria	100%/100%	Brazilian mangrove	red	circular	entire	raised
5	BQIII S-8	JX077095.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	tobacco	yellow	circular	erose	ex & crateri
6	BQIII S-9	JF701969.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	white	circular	entire	flat
7	BQIII S-11	JQ958882.1	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/100%	Aedes albopictus	gray	filamentous	filamentous	pulvinate
8	BQIII S-12	JN187857.1	*Streptomyces collinus*	Actinobacteria	Actinobacteria	100%/100%	marine sponge symbiont in sea water	white/yellow	filamentous	filamentous	flat
9	BQIII S-13(1)(2)	FN677987.1	*Paenibacillus lautus*	Firmicutes	Bacilli	100%/99%	gluten factory	clear	irregular	entire	flat
10	BQIII S-14(2)	FN677987.1	*Paenibacillus lautus*	Firmicutes	Bacilli	100%/99%	gluten factory	white	irregular	erose	flat
11	BQIII S-15	JF693986.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	white	irregular	entire	flat
12	BQIII S-17(2)	JF508413.1	*Paenibacillus sp.*	Firmicutes	Bacilli	100%/100%	desert soil	clear	irregular	lobate	flat
13	BQIII S-18	NR_044538.1	*Bacillus korlensis*	Firmicutes	Bacilli	100%/99%	desert soil sample	clear	punctiform	filamentous	flat
14	BQIII S-20	HQ143579.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	soil	cream	circular	filamentous	umbonate
15	BQIII S-23(1)	JX051309	*Streptomyces sp.*	Actinobacteria	Actinobacteria	100%/99%	coastal sediment	clear	filamentous	filamentous	flat
17	BQIII S-28(1)	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	cream	irregular	lobate	flat
18	BQIII S-29	JN791326.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	marine sediment	yellow	punctiform	entire	flat
19	BQIII S-30(1)	JF820115.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	oil field	yellow	circular	erose	umbonate
20	BQIII S-31	FR682744.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	clear	irregular	lobate	flat
21	BQIII S-32	AB552874.1	*Microbacterium sp.*	Actinobacteria	Actinobacteria	100%/99%	Algal-bacterial consortia	clear	punctiform	entire	flat
22	BQIII S-33	HQ285772.1	*Paenibacillus xylanilyticus*	Firmicutes	Bacilli	100%/99%	ice core samples	clear	irregular	erose	flat
23	BQIII S-34	JX077095.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	tobacco	clear	circular	entire	flat
24	BQIII S-34(1)	JX077095.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	tobacco	yellow	irregular	lobate	flat
25	BQIII S-35	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	seagrass	white	punctiform	entire	flat
26	BQIII S-38(2)	JN585695.1	*Microbacterium sp.*	Actinobacteria	Actinobacteria	100%/100%	rhizospheric soil of Arachis hypogaea	yellow	punctiform	entire	flat
27	BQIII S-39	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	seagrass	yellow	irregular	filamentous	flat
28	BQIII S-42	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	seagrass	yellow	irregular	lobate	flat
29	BQIII S-45	JF917313.1	*Isoptericola sp.*	Actinobacteria	Actinobacteria	100%/99%	seagrass	yellow	punctiform	entire	flat
30	BQIII S-47	JX077095.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	fresh leaf	yellow	circular	entire	convex
31	BQIII S-48	JX077095.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/100%	fresh leaf	white	irregular	erose	flat
32	BQIII S-51	AB425362.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	soil	pink	circular	entire	flat
33	BQIII S-53	JN215510.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/99%	salt	clear	filamentous	filamentous	flat
34	BQIII S-54	HQ647284.1	*Bacillus megaterium*	Firmicutes	Bacilli	100%/99%	rhizosphere	yellow	circular	erose	convex
35	BQIII S-57	JF701969.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	soil	yellow	irregular	lobate	flat
36	BQIII S-58	AB736321.1	*Bacillus sp.*	Firmicutes	Bacilli	100%/100%	deep-sea surface sediment	yellow	irregular	lobate	convex

Appendix I: Bacterial genera isolated from the mangrove soil and *Uca rapax*'s hindgut.

	Genera	Hindgut_Wet (%)	Hindgut_Dry (%)	Hindgut_Pre-sampling (%)	Soil_Wet (%)	Soil_Dry (%)	Nitrogen Fixing	References	Phosphate-solubilizing	References
1	*Bacillus sp.*	29.2	50.9	26.9	21.7	48.4	X	1, 4, 15	X	18, 6, 20, 22, 27, 29
2	*Microbacterium sp.*	17.5	1.7	9.2	15.1	1.6	X	10	X	19, 23, 24
3	*Streptomyces sp.*	10.0	9.5	0.0	0.0	5.6	X	2	X	20, 24
4	*Brevibacillus sp*	5.0	0.0	2.5	0.9	0.8				
5	*Cellulosimicrobium sp.*	5.0	0.0	1.7	1.9	0.0			X	21
6	*Pseudomonas sp.*	5.0	0.0	1.7	0.0	0.0	X	3, 4, 13	X	18, 6, 23, 26, 27, 29
7	*Gordonia sp.*	3.3	2.6	2.5	10.4	0.8			X	22
8	*Lysinibacillus sp.*	3.3	0.9	2.5	3.8	3.2	X	4		
9	*Paenibacillus sp*	3.3	5.2	0.0	0.0	4.0	X	4	X	6
10	*Paracoccus sp.*	2.5	0.0	0.0	0.0	0.0	X	5, 13		
11	*Vibrio sp.*	2.5	5.2	1.7	0.0	0.0	X	6, 13	X	6
12	*Achromobacter sp.*	1.7	0.0	0.0	0.0	0.0	X	7	X	18, 29
13	*Cellulomonas sp.*	1.7	0.0	3.4	1.9	0.0			X	23
14	*Isoptericola sp.*	1.7	7.8	1.7	10.4	2.4			X	24
15	*Klebsiella sp.*	1.7	0.0	0.0	0.0	0.0	X	6, 13	X	25
16	*Rhodococcus sp.*	1.7	0.9	0.0	2.8	0.8			X	22
17	*Bosea sp.*	0.8	0.0	0.0	0.0	0.0				
18	*Exiguobacterium sp.*	0.8	0.0	7.6	0.0	0.0			X	26
19	*Echinicola sp.*	0.8	0.0	0.0	0.0	0.0				
20	*Enterobacter sp.*	0.8	0.0	6.7	0.0	0.0	X	8	X	6, 26, 30
21	*Geobacillus sp.*	0.8	0.0	0.0	0.0	0.0				
22	*Serratia sp.*	0.8	0.0	0.0	0.0	0.0	X	4	X	22
23	*Microbulbifer sp.*	0.0	7.8	0.0	1.9	4.8				
24	*Halobacillus sp.*	0.0	3.4	0.0	0.0	15.9				
25	*Marinobacter sp.*	0.0	0.9	0.0	0.0	1.6				
26	*Ruegeria sp.*	0.0	0.9	0.0	0.0	0.8				
27	*Brachybacterium sp.*	0.0	0.9	0.0	0.0	0.0				
28	*Halomonas sp.*	0.0	0.9	0.0	2.8	0.0	X	9		
29	*Micrococcus sp.*	0.0	0.9	0.0	0.0	0.0			X	6, 27, 29, 30
30	*Proteus sp.*	0.0	0.0	9.2	0.0	0.0				
31	*Photobacterium sp.*	0.0	0.0	6.7	0.0	0.0				
32	*Aeromonas sp.*	0.0	0.0	5.0	0.0	0.0	X	13	X	27
33	*Citrobacter sp.*	0.0	0.0	2.5	0.0	0.0			X	28
34	*Acinetobacter sp.*	0.0	0.0	1.7	0.0	0.0			X	23, 26, 27
35	*Agromyces sp.*	0.0	0.0	1.7	0.0	0.0	X	10	X	24
36	*Shewanella sp.*	0.0	0.0	1.7	0.0	0.0				
37	*Delftia sp.*	0.0	0.0	0.8	0.0	0.0	X	11	X	22
38	*Dietzia sp.*	0.0	0.0	0.8	0.0	0.0				
39	*Sphingobacterium sp.*	0.0	0.0	0.8	0.0	0.0				
40	*Staphylococcus sp.*	0.0	0.0	0.0	5.7	0.0	X	12	X	23

Appendix I: Continuation

41	*Mycobacterium sp.*	0.0	0.0	0.0	3.8	0.8	X	10	X	29
42	*Oceanimonas sp.*	0.0	0.0	0.0	3.8	0.0	X	13		
43	*Serinicoccus sp.*	0.0	0.0	0.0	2.8	0.0				
44	*Sphingomonas sp.*	0.0	0.0	0.0	1.9	0.0	X	4		
45	*Aeromicrobium sp.*	0.0	0.0	0.0	0.9	0.0			X	30
46	*Agrococcus sp.*	0.0	0.0	0.0	0.9	0.0				
47	*Algoriphagus sp.*	0.0	0.0	0.0	0.9	0.0	X	14		
48	*Curtobacterium sp.*	0.0	0.0	0.0	0.9	0.0	X	15		
49	*Demequina sp.*	0.0	0.0	0.0	0.9	0.0				
50	*Nitratireductor sp.*	0.0	0.0	0.0	0.9	0.0	X	16		
51	*Nocardia sp.*	0.0	0.0	0.0	0.9	0.8	X	17		
52	*Novosphingobium sp.*	0.0	0.0	0.0	0.9	0.8	X	4		
53	*Planococcus sp.*	0.0	0.0	0.0	0.9	0.0				
54	*Arthrobacter sp.*	0.0	0.0	0.0	0.0	1.6	X	10, 13	X	22, 30
55	*Micromonospora sp.*	0.0	0.0	0.0	0.0	1.6	X	10	X	31
56	*Terribacillus sp.*	0.0	0.0	0.0	0.0	1.6				
57	*Nocardioides sp.*	0.0	0.0	0.0	0.0	0.8				
58	*Corynebacterium sp.*	0.0	0.0	0.0	0.0	0.8	X	10, 13	X	6, 27, 30
59	*Kocuria sp.*	0.0	0.0	0.0	0.0	0.8				

REFERENCES

1 Seldin, L., J. D. Van Elsas and E. G. C. Penido. 1984. Bacillus azotofixans sp. nov., a Nitrogen-Fixing Species from Brazilian Soils and Grass Roots. Int. J. Syst. Bacteriol. 34(4): 451-456.
2 Gadkari, D., G. Mörsdorf and O. Meyer. 1992. Chemolithoautotrophic assimilation of dinitrogen by Streptomyces thermoautotrophicus UBT1: identification of an unusual N2-fixing system. J. Bacteriol. 174(21): 6840-6843.
3 W. L. Barraquio, J. K. Ladha and I. Watanabe. 1983. Isolation and identification of N2-fixingPseudomonas associated with wetland rice. Can J Microbiol. 29(8):867-873.
4 Islam, R., P. Trivedi, M. Madhaiyan, S. Seshadri, G. Lee, J. Yang, Y. Kim, M. Kim, G. Han, P. Singh-Chauhan and T. Sa. 2010. Isolation, enumeration, and characterization of diazotrophic bacteria from paddy soil sample under long-term fertilizer management experiment. Biol Fert Soils. 46(3): 261-269.
5 Carlson, C. A. and J. L. Ingraham. 1983. Comparison of denitrification by Pseudomonas stutzeri, Pseudomonas aeruginosa, and Paracoccus denitrificans. Appl Environ Microbiol. 45(4): 1247–1253.
6 Thatoi, Hrudayanath, Bikash Chandra Behera, Rashmi Ranjan Mishra and Sushil Kumar Dutta. 2013. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. Ann Microbiol. 63:1–19.
7 Proctor, M. and P. W. Wilson. 1959. Nitrogen fixation by achromobacter spp. Archiv für Mikrobiologie. 32(3): 254-260.
8 Potrikus, C. J. and J. A. Breznak. 1977. Nitrogen-fixing Enterobacter agglomerans isolated from guts of wood-eating termites. Appl Environ Microbiol. 33(2): 392–399.
9 Argandoña, M., R. Fernandez-Carazo, I. Llamas, F. Martinez-Checa, J. M. Caba, E. Quesada and A. del Moral. 2005. The moderately halophilic bacterium Halomonas maura is a free-living diazotroph. FEMS Microbiology Letters. 244: 69–74.
10 Gtari, M., F. Ghodhbane-Gtari, I. Nouioui, N. Beauchemin and L.S. Tisa . 2012. Phylogenetic perspectives of nitrogen-fixing actinobacteria. Arch Microbiol. 194(1): 3-11.
11 Han, J., L. Sun, X. Dong, Z. Cai, X. Sun, H. Yang, Y. Wang and W. Song. 2005. Characterization of a novel plant growth-promoting bacteria strain Delftia tsuruhatensis HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Syst Appl Microbiol. 28(1): 66-76.
12 Holguin, G., M. A. Guzman and Y. Bashan. 1992. Two new nitrogen-fixing bacteria from the rhizosphere of mangrove trees: Their isolation, identification and in vitro interaction with rhizosphere Staphylococcus sp. FEMS Microbiology Ecology. 101: 207-216.
13 Liu, J., M. Peng and Y. Li. 2012. Phylogenetic diversity of nitrogen-fixing bacteria and the nifH gene from mangrove rhizosphere soil. Can J Microbiol. 58(4): 531-539.
14 Severin, I., S. G. Acinas and L. J. Stal. 2010. Diversity of nitrogen-fixing bacteria in cyanobacterial mats. FEMS Microbiol Ecol. 73(3): 514-525.
15 Sturz, A. V., B. R. Christie, B. G. Matheson and J. Nowak. 1997. Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol Fert Soils. 25(1): 13-19.
16 Liu, M., L. Fan, L. Zhong, S. Kjelleberg and T. Thomas. 2012. Metaproteogenomic analysis of a community of sponge symbionts. ISME J. 6(8): 1515-1525.
17 Metcalfe, G. and M. E. Brown. 1957. Nitrogen Fixation by New Species of Nocardia. Microbiology. 17(3): 567-572.
18 Rodríguez H. and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv. 17(4-5): 319-339.
19 Rivas, R., M.E.,Trujillo, M. Sánchez, P.F., Mateos, E. Martínez-Molina and E. Velázquez. 2004. Microbacterium ulmi sp. nov., a xylanolytic, phosphate-solubilizing bacterium isolated from sawdust of Ulmus nigra. Int J Syst Evol Microbiol. 54(2): 513-517.
20 Banik, S., B. K. Dey. 1982. Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing micro-organisms. Plant and Soil. 69(3): 353-364.
21 Liu, C.H., J.Y. Wu and J.S. Chang. 2008. Diffusion characteristics and controlled release of bacterial fertilizers from modified calcium alginate capsules. Bioresour Technol. 99(6):1904-1910.
22 Chen, Y.P., P.D. Rekha, A.B. Arun, F.T. Shen, W.-A. Lai and C.C. Young. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology. 34: 33–41.
23 QianYing, C., H. ZiQuan, L. LianSheng, X. NaNa and Z. HaiQuan. 2009. Isolation and identification of six phosphate-solubilizing bacteria. Journal of Anhui Agricultural University. 36(3): 417-421.
24 Dastager, Syed G. and S. Damare. 2013. Marine Actinobacteria Showing Phosphate-Solubilizing Efficiency in Chorao Island, Goa, India. Current Microbiology. 66(5): 421-427.
25 Chung, H., M. Park, M. Madhaiyan, S. Seshadri, J. Songb, H. Cho and T. Sa. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biology and Biochemistry. 37(10): 1970–1974.
26 Collavino, M., P. A. Sansberro, L. A. Mroginski and O. Mario Aguilar. 2010. Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote Phaseolus vulgaris growth. Biology and Fertility of Soils. 46(7): 727-738.
27 Barea J.M., E. Navarro and E. Montoya. 2008. Production of Plant Growth Regulators by Rhizosphere Phosphate-solubilizing Bacteria. J Appl Bacteriol. 40(2):129-134.
28 Gyaneshwar, P., G. Naresh Kumar and L.J. Parekh. 1998. Effect of buffering on the phosphate-solubilizing ability of microorganisms. World Journal of Microbiology and Biotechnology. 14(5): 669-673.
29 Guiñazú, L. B., J. A. Andrés, M. F. Del Papa, M. Pistorio and S. B. Rosas. 2010. Response of alfalfa (Medicago sativa L.) to single and mixed inoculation with phosphate-solubilizing bacteria and Sinorhizobium meliloti. Biology and Fertility of Soils. 46(2): 185-190.
30 Martínez-Nieto, P and D. G. García-González. 2010. Bacterias diazotroficas y solubilizadoras de fósforo aisladas de las especies forestales altoandinas colombianas Weinmannia tomentosa y Escallonia myrtilloides. Revista del Instituto de Investigaciones Tropicales. 5: 63-76.
31 El-Tarabily, K. A., A. H. Nassar and K. Sivasithamparam. 2008. Promotion of growth of bean (Phaseolus vulgaris L.) in a calcareous soil by a phosphate-solubilizing, rhizosphere-competent isolate of Micromonospora endolithica. Applied Soil Ecology. 39(2): 161-171.