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Bacterial flora associated with commercial *Octopus maya* captured in the Yucatan Peninsula, Mexico

Flora bacteriana asociada al *Octopus maya* comercial capturado en la Península de Yucatán, México

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ABSTRACT

Background: Octopus is a fishery product of economic importance worldwide, the main species caught on the coast of the Gulf of Mexico and the Caribbean Sea are *Octopus maya* and *O. vulgaris*, the first represents up to 95 % of national production. **Goals**: Identify the bacterial flora associated with commercial *Octopus maya* captured in the Yucatan Peninsula, using PCR-DGGE. **Methods**: From the metagenomic DNAs (mDNAs) extracted from samples representative of the octopus muscle, PCR products were synthesized with universal primers for bacteria (gc338F and 518R) and specific primers for Phylum Firmicutes (FirF: 369 and gcFirR: 1244). They were separated by electrophoresis in denaturing gradient gels (DGGE). The fragmented DNAs were recovered by elution, amplified (338F / 518R and FirF: 369 / FirR: 1244), sequenced and analyzed phylogenetically. **Results**: The sequences amplified with universal primers, after the DNA fragmentation by DGGE were associated with *Psychrobacter urativorans*, *Psychrobacter* sp, *Pseudomonas* sp, *Pseudoalteromonas* sp, *Shewanella* sp, *Shewanella baltica*, *Klebsiella oxytoca, Vibrio aestuarianus*, *Photobacterium* sp*, Flavobacterium* sp, *F. antarcticum*, *Bizionia* sp, *Flavobacteriaceae bacterium*, *Bacillus* sp, *C. divergens*, *Cetobacterium somerae*, *Psychrilyobacter atlanticus*, *Salinimicrobium* sp as well as, *Flavobacteriaceae* not yet classified. In the sequences amplified with specific primers (Phylum Firmicutes) were identified: *Carnobacterium* sp, *Lactococcus piscium Lactococcus* sp, and *Vagococcus* sp **Conclusion**: The bacterial genus detected have been reported in samples from marine environments; therefore, can be part of the native microbial diversity associated with commercial *O. maya* captured in the Yucatan Peninsula, Mexico.

Keywords: Bacterial flora, Octopus, PCR-DGGE, phylotypes

RESUMEN

Antecedentes: El pulpo es un producto pesquero de importancia económica a nivel mundial, las principales especies capturadas en el litoral del Golfo de México y el mar Caribe son *Octopus maya* y *O. vulgaris*, el primero representa hasta el 95 % de la producción nacional. **Objetivo**: Identificar la flora bacteriana asociada al *Octopus maya* comercial capturado en la Península de Yucatán, utilizando PCR-DGGE. **Métodos**: A partir de los ADN metagenómicos (ADNmg) extraídos de muestras representativas del m**ú**sculo del pulpo, se sintetizaron productos de PCR con iniciadores universales para bacterias (gc338F y 518R) e iniciadores específicos para el filo Firmicutes (FirF:369 y gcFirR: 1244). Mismos que fueron separados por electroforesis en geles de gradiente desnaturalizante (DGGE). Los ADN fragmentados se recuperaron por elución, se amplificaron (338F / 518R y FirF: 369 / FirR: 1244), se secuenciaron y analizaron filogenéticamente. **Resultados**: Las secuencias amplificadas con iniciadores universales, después de la fragmentación del ADN por DGGE se asociaron con *Psychrobacter urativorans*, *Psychrobacter* sp, *Pseudomonas* sp, *Pseudoalteromonas* sp, *Shewanella* sp, *Shewanella baltica*, *Klebsiella oxytoca, Vibrio aestuarianus*, *Photobacterium* sp*, Flavobacterium* sp, *F. antarcticum*, *Bizionia* sp, *Flavobacteriaceae bacterium*, *Bacillus* sp, *Carnobacterium divergens*, *Cetobacterium somerae*, *Psychrilyobacter atlanticus, Salinimicrobium* sp, así como, *Flavobacteriaceae* aún no clasificada. En las secuencias amplificadas con iniciadores específicos (filo Firmicutes) se identificaron: *Carnobacterium* sp, *Lactococcus piscium, Lactococcus* sp y *Vagococcus* sp. **Conclusión**: Los géneros bacterianos detectados han sido reportados en muestras de ambientes marinos, por lo cual, pueden ser parte de la diversidad microbiana nativa asociada al *O. maya* comercial capturado en la Península de Yucatán, México.

Palabras clave: Flora bacteriana, pulpo, PCR-DGGE, filotipos

INTRODUCTION

In 2014, Mexico ranked 16th among the main countries producing fish resources worldwide. A wide range of fisheries are exploited in the country, ranging from octopus, tuna, shrimp, lobster, squid, and sardine; Spain, Italy, and Japan are the largest consumers, and importers of these fishes' species. Morocco and Mauritania are the principal Octopus exporters (FAO, 2014). In Mexico, the states of Campeche and Yucatan represent 94 % of the national catch, which in 2016 was more than 38 thousand tons. Currently, Mexico is positioned in the third octopus producer in the world (CONAPESCA, 2017). Fishery products are highly susceptible to microbial growth due to the chemical composition (proteins, moisture, and lipids), aquatic habitat and post-harvest handling (Adedeji *et al.*, 2012). Microorganisms can have both positive and negative effects on food industry systems. Many are used as probiotic agents, quality indicators and flavor enhancers (de la Cruz-Leyva *et al.*, 2015) or as elements in a variety of biotechnological processes (Hernández *et al.*, 2016). Others negatively affect product quality by acting as deteriorating agents. A large proportion of gastroenteritis and food poisoning cases worldwide are caused by ingestion of uncooked fishery products contaminated with pathogenic agents (Silveira, 2016).

Plate culture and biochemical tests are the most common methods for microbial analysis and identification in different food matrices, even though results document only a minority of the microbial community (Kirkup, 2013). In recent years, a number of culture independent methods have come into use in which DNA samples are used to amplify the rRNA 16S gene by PCR (Nübel *et al.*, 1999), and then analyzed using genetic fingerprint methods such as denaturing gradient gel electrophoresis (DGGE) (de la Cruz-Leyva *et al.*, 2011), Temperature gradient gel electrophoresis (TGGE), Restriction fragment length polymorphism (RFLP), Ribosomal intergenic spacer analysis (RISA) and Automated ribosomal intergenic spacer analysis (ARISA) and third generation sequencing methods. These methods have allowed the characterization of culturable and unculturable bacterial populations in different complex samples (Fakruddin & Mannan, 2013). The DGGE method is a simple genetic fingerprint method able to separate small DNA fragments of the same size but with different sequences (Muyzer & Smalla, 1998). It has been widely used for identifying bacterial flora in environmental samples and applied to study microbial communities in fishery resources attention (Wu *et al.*, 2012), although cephalopods have received little attention.

The octopus fishery is significant economic activity on the coasts of the Yucatan Peninsula, Mexico. Octopus has recently served as a model for research addressing population density and dynamic (Gamboa-Álvarez *et al.*, 2015), physiology (Martínez *et al.*, 2014; Rodríguez-Domínguez *et al.*, 2013), development (Noyola *et al.*, 2013), feeding (Linares *et al.*, 2015), nutrition (Tercero *et al.*, 2015), and captive breeding and production (Baeza-Rojano *et al.*, 2013). No research has been done to date on the bacterial community associated with this resource, this information is essential to establishing adequate quality controls for the detection of undesirable microflora during product storage, processing, and marketing. The goal of this study was to characterize the bacterial flora associated with commercial *Octopus maya* captured in the Yucatan Peninsula, using PCR-DGGE.

MATERIALS AND METHODS

Sampling. In this work, 36 commercial samples of *O. maya* were collected in the reception area of a fish products packing company located in Merida Yucatan, Mexico; following the Mexican regulations for the capture, handling, and transportation of this samples (NOM-109-SSA1-1994). Criteria in the selection of the samples: that the specimens did not have more than six hours of capture and that the cold chain had been maintained, from its capture to the packing company of fishery products. All specimens were captured on the coast of the Gulf of Mexico (August to December 2008), located in the Yucatan Peninsula. It occupies a territory of approximately 125,000 km², is located in southeastern Mexico and divides the Gulf of Mexico from the Caribbean Sea in the extreme southeast of North America and northern Central America.

The octopus samples collected in the processing plant of fishery products, were transferred to the laboratory at -10 ºC in a time not exceeding 60 min, in the laboratory were stored at -20 °C until use.

Sample preparation. 20 G of the surface tissue of each sample was mixed with 1X TEN buffer (100 mM Tris-HCl pH 8.0, 500 mM NaCl, 50 mM EDTA pH 8.0) at a 1:1 ratio and homogenized for 1 min a vortex with the highest setting. A 1500 µl aliquot was taken and centrifuged at 16270 x g for 8 min at 28 ºC, the supernatant discarded and the pellet suspended in 1 ml 1X TEN buffer. This mixture was centrifuged at 16270 x g for 5 min at 28 ºC, and the pellet was stored at -20 °C until use.

DNA extraction. Extraction of mgDNA was done following a silica-based method described by Rojas-Herrera *et al.* (2008). Initially, 1 ml of 1X TEN buffer was added to the pellet and vigorously shaken for 15 sec. Then, 10 µl of 10 % lysozyme (10 mg lysozyme/ml TE, 10 mM Tris-HCl and 1 mM EDTA, pH 8.0) was added and the solution incubated for 30 min at 37 °C with gentle mixing every 10 min. The solution was then cooled by placing in an ice-alcohol bath for 10 min and then incubated for 5 min at 65 °C, a process which was repeated three times. After the third repetition, 100 µl of 20 % (w/v) SDS were added, the solution was agitated for 15 sec by manual inversion, incubated for 20 min at 30 °C and centrifuged for 10 min under the above conditions. A total of 400 µl of 5 M potassium acetate were added to the supernatant, the solution was incubated for 5 min at 65 °C and then placed in an ice-alcohol bath for 20 min. The solution was centrifuged at 16270 x g for 15 min at 10 °C. The aqueous phase was transferred to a sterile tube, 200 µl of 4 % (w/v) silicium dioxide (SiO_2) was added, the tube was agitated for 3 min by inversion and centrifuged at 16270 x g for 2 min at 28 °C. The resulting pellet was washed twice with 1 ml of 70 % ethanol, centrifuged each time under the above conditions and the alcohol residues evaporated. The pellet was then suspended in 50 µl sterile distilled water and incubated for 5 min at 55 °C with agitation every 1 min. This suspension was centrifuged for 2 min under the above conditions, the aqueous phase recovered without disturbing SiO_2, and the extracted DNA stored at -20 °C until use. The DNA presence was verified by 1 % (w/v) agarose gel electrophoresis dyed with ethidium bromide (0.3 µg/ml) together with a carrier solution (1:1 ratio). Lambda DNA/Hind III ladder molecular marker was used as a reference. Electrophoresis was run with 1X TAE buffer (50X stock solution: 24.2 % Tris base, 5.71 % glacial acetic acid, 3.72 % $Na_2EDTA2H_2O$) for 2 h at 60 V. Gels were viewed under a UV transilluminator and the image stored in an imager (Gel Doc XR system, BioRad) using the Quantity One program (BioRad Imaging

Systems). To estimate DNA purity and concentration, it was diluted in sterile, ion-free water (ddH_2O) at a 1:250 ratio and absorbance read at 260 and 280 nm in a spectrophotometer.

DNA amplification. The V3 region of the 16S rRNA gene was amplified using the primers gc338F (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCTCCTAC GGGAGGCAGCAG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3') (Muyzer *et al*., 1993). The underlined sequence in the gc338F primer corresponds to the GC clamp. The reaction mixture contained 100 ng DNA sample, 2.5 µl 1X PCR buffer, 0.2 mM dNTPs mixture (Invitrogen, Carlsbad, CA), 0.2 mM of each primer, 4.5 mM $MgCl_2$ (Promega), 0.1 % bovine serum albumin (BSA) and 1 U Taq polymerase DNA (Invitrogen™). The PCR run was as follows: denaturation, 94 °C for 5 min (1 cycle); touchdown at 10 °C above alignment temperature (65 °C), temperature lowered by 1 °C/min until 55 °C and increased to 72 °C for 1 min (10 cycles); denaturation, 94 °C for 1 min; alignment, 55 °C for 1 min; extension, 72 °C for 1 min (20 cycles); final extension, 72 °C for 10 min. The Phylum Firmicutes 16S rRNA fragments were amplified using the primers FirF: 369 (5'-GGAGGCAGCAGTAGGNAATCTTC-3') and gcFirR: 1244 (5'-CGCCCGCCGCGCGCGG CGGGCGGGGCGGGGGCACGGGGGGTAGCCCARGTCATAAGGGGCATG-3') (850 pb) (Rojas-Herrera *et al*., 2008). The reaction mixture contained 100 ng DNA sample, 4.5 mM $MgCl_2$, 0.1 % BSA, 2.5 µl 5X PCR buffer, 0.2 mM dNTPs, 0.2 mM of each primer, 1 U Taq polymerase (Promega®) and sterile double-distilled water to complete final reaction volume to 25 µl. The run was as follows: denaturation, 94 °C for 5 min (1 cycle); amplification, 94 °C for 40 sec, alignment at 63 °C for 60 sec, extension at 72 °C for 30 sec (35 cycles); final extension at 72 °C for 7 min (1 cycle). Amplicon verification was done with 1.8 % agarose gel for the V3 region and with 1.5 % agarose gel for the Phylum Firmicutes fragments, using the conditions described in the previous section, except for the use of 0.5X Tris borate-EDTA (TBE) as the electrophoresis run buffer.

DGGE. Amplicon separation was done by DGGE with a Dcode™ Universal Mutation Detection System (BioRad), following manufacturer instructions and the protocol described by Muyzer *et al.* (1993). The 200 bp amplicons were fragmented in 8 % polyacrylamide gel (w/v) with a 30 to 60 % urea formamide denaturing gradient (100 % corresponds to 7 M urea and 40 % deionized formamide (w/v). For each well 75 µl (500 ng) were mixed with 30 µl 5X load buffer. Electrophoresis was run in 1X TAE buffer at 60 V for 22 h at 60 °C. Firmicutes (900 bp) fragments separation were done in 6 % polyacrylamide gel (w/v) with a 35 to 60 % urea-formamide denaturizing gradient. Amplicon load volume and electrophoresis conditions were as described above.

Gels were dyed with a 0.5X SYBR GOLD solution (Invitrogen™, Eugene, Oregon, USA) (5 µl/100 ml 1X TAE buffer) for 45 min and viewed in a UV transilluminator. Images were stored as described previously. Unique bands in the banding patterns were cut and eluted for 16 h in 50 µl sterile double-distilled water at 4 °C. The eluted DNA was amplified by PCR using the previously described conditions, with the exception of the addition of BSA and $MgCl_2$ (3 mM for universal fragments, 4 mM for Phylum Firmicutes fragments). The amplicons derived from the cut and DGGE eluted bands were sequenced at an outside laboratory (Macrogen, South Korea).

Sequence analysis. Management and refinement of the universal (±150 bp) and Phylum Firmicutes (±650 bp) fragments were done with the BioEdit program, while taxonomic classification was done with the BLAST program with the Ribosomal Database Project (RDP) and GenBank databases. Nearest neighbors were calculated in the RDP database (Classifier and Sequence Match tools, ver. 10.0) (Cole *et al*., 2003; Cole *et al*., 2005). A phylogenetic association was observed using a maximum parsimony tree with a 100 bootstrap test run applied with the WinClada program (Nixon, 1999), and including *Micrococcus luteus* as an outgroup.

RESULTS

The DNA isolated from the 36 *O. maya* samples exhibited high molecular weight (average 23,130 kb) with an average purity and concentration of 1.1294 ± 39 and 99 ± 16 µg/ml respectively. The phylogenetic study of the bacterial flora associated with *O. maya* samples was developed from 25 of the 37 sequences obtained because 12 of them exhibited noisy sequencing and low-quality chromatograms.

Taxonomic classification showed 60 % belongs to Phylum Proteobacteria, 24 % to Bacteroidete*s* (consisting of *Cytophaga-Flexibacter-Bacteroides* [CFB] group) and 8 % each with Phylum Fusobacteria and Firmicutes (Table 1). For the 25 partial DNA sequences (±150 bp) describing overall bacterial flora, the phylogenetic association was determined by applying maximum parsimony with the 16S rRNA gene sequences for the 31 nearest neighbors as calculated with the RDP; 16S rRNA gene sequences for *M. luteus* (*Actinobacteria*) were used as the outgroup.

Only 19.84 % (775) of the 3907 -character alignment was phylogenetically informative and these characters generated a total of 16 equally parsimonious trees (L=3039) (figure not shown).

This phylogenetic grouping showed the bacterial flora associated with the Octopus samples to be composed mainly of Gammaproteobacteria: *Psychrobacter urativorans* (YucOct24), *Psychrobacter* sp (YucOct25 and YucOct27), *Pseudomonas* sp (YucOct19), *Pseudoalteromonas* sp (YucOct21 and YucOct20), *Shewanella* sp (YucOct4 and YucOct26), *S. baltica* (YucOct3 and YucOct5), *K. oxytoca* (YucOct28), *Vibrio aestuarianus* (YucOct11) and *Photobacterium* sp (YucOct8, YucOct9 and YucOct10). Of secondary importance were phylotypes related to the CFB group: *F. antarcticum* (YucOct23), *Flavobacterium* sp (YucOct17), *Bizionia* sp (YucOct13 and YucOct14), *Salinimicrobium* sp (YucOct1), and uncultured *Flavobacteriaceae* (YucOct18). In a minor proportion, sequences were also identified affiliated as *Cetobacterium somerae* (YucOct6) and *Psychrilyobacter atlanticus* (YucOct16) (Phylum Fusobacteria). The species *Bacillus* sp (YucOct12) and *C. divergens* (YucOct7) were affiliated with Phylum Firmicutes.

Of the 30 bands eluted from the banding profiles describing the Fhylum Firmicutes only 17 could be amplified. The exact cause is still unclear, although it may be related to fragment size (900 bp) since one of DGGE's limitations is its separation of relatively small (200 to 700 bp) fragments (Muyzer *et al*., 1998). As a result, the phylogenetic study was done using the recovered and sequenced fragments. The Bayesian analysis implemented with the RDP database classifier tools showed most of these sequences belong to the *Carnobacteriaceae* family (53 %), followed by the *Streptococcaceae* (35.2 %) and *Enterococcaceae* (11.8 %) families (Table 2). An affiliation of the 17 partial Phylum Firmicutes group sequences (±650 bp) recovered from the DGGE fragments was done by phylogenetic analysis with the maximum parsimony method using the 16S rRNA gene sequences for the 20 nearest neighbors as calculated in the RDP; 16S rRNA gene sequences for *M. luteus* were

used as the outgroup. Of the 1312 characters in the alignment, only 26.2 % (428) were phylogenetically informative. The maximum parsimony tree (L=998) showed that 59 % of the bacterial flora associated with the *Octopus* samples matched Phylum Firmicutes (figure not shown): 47 % *Carnobacterium* sp (MaCCL1, MaCCL2, MaCCL6, MaCCL7, MaCCL12, MaCCL13, MaCCL14, and MaCCL15) and 6 % uncultured *bacterium* (MaCCL3). A further 35.2 % of the analyzed sequences matched *Lactococcus piscium* (MaCCL8 and MaCCL9), *Lactococcus* sp (MaCCL4, MaCCL5, MaCCL10, and MaCCL11), and two phylotypes were identified as *Vagococcus* sp (MaCCL16 and MaCCL17). This phylogenetic association analysis of the recovered and analyzed bacterial mgDNA sequences extracted from commercial Octopus samples produced the description of the bacterial flora associated with this fishery product.

DISCUSSION

Genetic fingerprint molecular techniques have been proposed for the study of the structure of bacterial communities associated with complex samples; including microorganisms that have not yet been cultured in the laboratory (Piterina & Pembroke, 2013). Use of universal PCR products in DGGE analysis allows for detection of 90 to 99 % of numerous species in a community, which is why specific primers are needed to study specific microorganisms (Mühling *et al.*, 2008).

From the phylogenetic analysis of the sequences recovered during this investigation, part of the bacterial flora associated with the *O. maya* was observed, which were grouped to Phylum Proteobacteria (60 %), Bacteroidetes (24 %), Fusobacteria and Firmicutes (the last two 8 % each). This it coincides with the detected previously, during the stan-

Table 1. Taxonomic classification of 25 sequences amplified with universal primers (338F / 518R) recovered from elution of fragment DNA by PCR-DGGE[a].

Phylotype	GenBank Accession Number	Phylum	Genus or species	Similarity (%)[b]
YucOct1	HM007325	Bacteroidetes	*Salinimicrobium* sp	94
YucOct3	HM007326	Proteobacteria	*Shewanella baltica*	100
YucOct4	HM007327	Proteobacteria	*Shewanella* sp	98
YucOct5	HM007328	Proteobacteria	*S. baltica*	100
YucOct6	HM007329	Fusobacteria	*Cetobacterium somerae*	97
YucOct7	HM007330	Firmicutes	*Carnobacterium* sp	100
YucOct8	HM007331	Proteobacteria	*Photobacterium* sp	98
YucOct9	HM007332	Proteobacteria	*Photobacterium* sp	99
YucOct10	HM007333	Proteobacteria	*Photobacterium* sp	97
YucOct11	HM007334	Proteobacteria	*Vibrio aestuarianus*	97
YucOct12	HM007335	Firmicutes	*Bacillus* sp	93
YucOct13	HM007336	Bacteroidetes	*Bizionia* sp	97
YucOct14	HM007337	Bacteroidetes	*Bizionia* sp	99
YucOct16	HM007338	Fusobacteria	*Psychrilyobacter atlanticus*	97
YucOct17	HM007339	Bacteroidetes	*Flavobacterium* sp	99
YucOct18	HM007340	Bacteroidetes	Uncultured *bacterium*	95
YucOct19	HM007341	Proteobacteria	*Pseudomonas* sp	100
YucOct20	HM007342	Proteobacteria	*Pseudoalteromonas* sp	100
YucOct21	HM007343	Proteobacteria	*Pseudoalteromonas* sp	100
YucOct23	HM007344	Bacteroidetes	*Flavobacterium antarcticum*	100
YucOct24	HM007345	Proteobacteria	*Psychrobacter urativorans*	99
YucOct25	HM007346	Proteobacteria	*Psychrobacter* sp	98
YucOct26	HM007347	Proteobacteria	*Shewanella* sp	96
YucOct27	HM007348	Proteobacteria	*Psychrobacter* sp	96
YucOct28	HM007349	Proteobacteria	*Klebsiella oxitoca*	98

[a] The DNA was extracted from samples of commercial *O. maya* captured on the coast of the Gulf of Mexico (Yucatan Peninsula).

[b] Last taxonomic range with which the sequence has a similarity equal to or greater than 80 %, based on the RDP Bayesian classifier.

[c] Without classification in this range. Sequences with less than 80 % similarity with the following range.

dardization of an RNA isolation and purification method for the study of the bacterial flora of *O. maya* samples; where the Phylum Proteobacteria (75 %), including the families *Vibrionaceae* and *Enterobacteriaceae*, were those that were identified mainly (de la Cruz-Leyva *et al.*, 2011). In a study conducted in *O. variabilis*, they also reported the presence of Phylum Proteobacteria (63.5 %), Bacteroidetes (18.6 %) and Firmicutes (3.5 %), among others, using Illumina Miseq sequencing and quantified by real-time PCR (Lee *et al.*, 2017).

The octopus has largely benthic habits, which keep it in constant contact with marine sediments; which may, in turn, explain the link between marine and sedimentary ecosystems in its bacterial community diversity. In marine sediments have been detected Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes, and dominated mainly by the genus *Pseudoalteromonas* (40.5 %), *Bacillus* (36.3 %) and *Photobacterium* (5.8 %) (Li *et al.*, 2017). It has also been reported that the diversity of microbiota and bacterial loads in octopuses is influenced by environmental factors such as water temperature and geographic location (Lee *et al.*, 2017). The sex of the animal could also influence the bacterial diversity contained in Octopus samples. It has been mentioned that the predominant species in the female octopus are *Vibrionaceae* and *Streptococcaceae*, whereas only *Vibrionaceae* were identified more frequently in male octopuses (Lehata *et al.*, 2015). In this research, the presence of *V. aestuarianus* was also detected in 56 % of the octopus samples analyzed, this bacteria has been isolated from mussels, plankton, sediments, and seawater. It is one of the main pathogens in the culture of Pacific oysters *Crassostrea gigas* in France, Spain, and Italy, causing considerable economic losses (Travers *et al.*, 2017). Therefore, it is corroborated that the bacterial diversity detected in this study is consistent with the microflora described in environments, resources and marine products.

It has been mentioned that lactic acid bacteria (LAB) make up only a small proportion of any bacterial community, which makes it difficult to detect when PCR is used with universal primers, for example in human feces (Walter *et al.*, 2001).

In the present work, two LAB (*Bacillus* sp and *C. divergens*) were detected from universal primers. However, with specific primers (Phylum Firmicutes) 17 sequences related to LAB (*Carnobacterium* sp, *L. piscium*, *Lactococcus* sp, and *Vagococcus* sp) were identified in *O. maya*.

Genetic fingerprint methods allow the identification of all the microorganisms in fishery resources, both those acquired in the environment and during handling, storage and export. For this reason, the PCR-DGGE method is recommended as a traceability tool. Traceability studies allow the detection of functional microorganisms with biotechnological potential; as well as the timely detection of spoilage bacteria in fishery products and identification of pathogenic microorganisms that can affect the health of consumers.

Table 2. Taxonomic classification of 17 sequences amplified with primers for Phylum Firmicutes (FirF: 369 / FirR: 1244) recovered from elution of fragment DNA by PCR-DGGE[a].

Phylotype	GenBank Accession Number	Family	Genus or species	Similarity (%)b
MaCCL1	HM007308	*Carnobacteriaceae*	*Carnobacterium* sp	99
MaCCL2	HM007309	*Carnobacteriaceae*	*Carnobacterium* sp	100
MaCCL3	HM007310	*Carnobacteriaceae*	*Carnobacterium* sp	100
MaCCL4	HM007311	*Streptococcaceae*	*Lactococcus* sp	96
MaCCL5	HM007312	*Streptococcaceae*	*Lactococcus* sp	100
MaCCL6	HM007313	*Carnobacteriaceae*	*Carnobacterium* sp	99
MaCCL7	HM007314	*Carnobacteriaceae*	*Carnobacterium* sp	99
MaCCL8	HM007315	*Streptococcaceae*	*Lactococcus piscium*	100
MaCCL9	HM007316	*Streptococcaceae*	*Lactococcus piscium*	99
MaCCL10	HM007317	*Streptococcaceae*	*Lactococcus* sp	99
MaCCL11	HM007318	*Streptococcaceae*	*Lactococcus* sp	98
MaCCL12	HM007319	*Carnobacteriaceae*	*Carnobacterium* sp	99
MaCCL13	HM007320	*Carnobacteriaceae*	*Carnobacterium* sp	98
MaCCL14	HM007321	*Carnobacteriaceae*	*Carnobacterium* sp	96
MaCCL15	HM007322	*Carnobacteriaceae*	*Carnobacterium* sp	93
MaCCL16	HM007323	*Carnobacteriaceae*	*Vagococcus* sp	97
MaCCL17	HM007324	*Carnobacteriaceae*	*Vagococcus* sp	87

[a] The DNA was extracted from samples of commercial *O. maya* captured on the coast of the Gulf of Mexico (Yucatan Peninsula).

[b] Last taxonomic range with which the sequence has a similarity equal to or greater than 80 %, based on the RDP Bayesian classifier.

This capacity is important for identifying particularly troublesome strains such as LAB. These have become problematic in aquaculture systems during the recent decades because they contain pathogenic strains which have caused infection and death in fish worldwide. Other LAB species identified from fish and *O. vulgaris* include *L. garvieae* (Fichi *et al*., 2015; Meyburgh *et al*., 2017), *L. raffinolactis*, *Streptococcus iniae, S. dysgalactiae*, *S. parauberis*, *S. agalactiae*, *Carnobacterium* sp, *Enterococcus* "*faecium*" group, *Vagococcus fluvialis*, *V. carniphilus*, *V. salmoninarum*, and *Aerococcus* sp. Most of them are an opportunistic species that cause infections in fish and crustaceans (Michel *et al*., 2007). The genus *Carnobacterium*, *Lactococcus* and *Vagococcus* were detected in this work.

On the other hand, it is important to mention that species of this genus have been suggested as probiotics in aquaculture (Lebreton *et al*., 2013; Sequeiros *et al*., 2015). Of particular interest is the identification of the LAB genus *Carnobacterium*, especially the species *C. divergens*, since it can tolerate high pressure (piezophile), freezing and thawing, also has properties anaerobic. *Carnobacterium* strains are commonly isolated from a variety of environments, vacuum-packed and modified-atmosphere packed (MAP) meats, and seafood products (e.g. cod, salmon and shrimp) (Macé *et al*., 2013). For example, in a study of aerobic and anaerobic microbial communities of MAP salmon and coalfish by using multivariate analysis of the 16S rRNA gene with RT-PCR and T-RFLP, a strong association was observed between *Brochothrix thermosphacta* and *C. divergens* with salmon (Rudi *et al*., 2004). However, this genus can also metabolize arginine and various carbohydrates, including chitin. It has been studied widely as a growth inhibitor for *Listeria monocytogenes* in fish and meat products and for its production of bacteriocins and tyramine, which inhibit deteriorating microflora (Leisner *et al*., 2007). Study of the total microbial diversity in fishery products generate vital data for the control of food production; as well as the hazard analysis critical control point (Rudi *et al*., 2004).

In general, the microorganisms of marine ecosystems are considered extremophiles, because they can withstand high or low temperatures, halotolerant, withstand high hydrostatic pressures, alkaline pH and even anoxic conditions; they have been studied extensively for having the capacity to produce enzymes, antimicrobial metabolites such as bacteriocins, exopolysaccharides (EPS), among others (Poli *et al*., 2017). The properties of extremophile microorganisms are desirable since they can be used in biotechnological processes. In this sense, it is important to mention that of the genus or species detected in this work from the amplification with universal primers (*P. urativorans, Psychrobacter* sp, *Pseudomonas* sp, *Pseudoalteromonas* sp, *Shewanella* sp, *S. baltica*, *K. oxytoca*, *V. aestuarianus*, *Photobacterium* sp, *Flavobacterium* sp, *F. antarcticum*, *B. paragorgiae*, *Salinimicrobium* sp, *Bacillus* sp, *C. divergens, Cetobacterium somerae*, *Psychrilyobacter atlanticus*, and uncultured *Flavobacteriaceae*), there are some genus that has been reported with some biotechnological characteristic. For example, species of the genus *Photobacterium* are psychrophiles, piezophile (*P. profundum*) and bioluminescent for example *P. phosphoreum*, some species live in symbiosis with marine organisms (*P. leiognathi*) (Urbanczyk *et al*., 2011). The genus *Pseudomonas* possesses a diversity of bacterial species of medical (pathogenic strains: *P. aeruginosa*, *P. fluorescens*, among others) and biotechnology importance, has been isolated from different habitats (such as soils, fresh or marine water, plants, animals, biofilms, plankton, among others), thanks to its metabolic versatility (Özen & Ussery. 2012). Exopolysaccharides (EPS) is produced by bacteria of the genus *Pseudomonas*, *Pseudoalteromonas*, *Shewanella*, *Klebsiella* to adapt and remain in diverse environmental niches. These biopolymers are of interest due to their involvement in the formation of biofilms, the formation of capsules, virulence or their possible medical and industrial uses (Hay *et al*., 2014; Roca *et al*., 2016). Recently it was observed that EPS have the capacity to absorb heavy metals (mercury and cadmium) (Caruso *et al*., 2018); *K. oxytoca* and *Shewanella* sp, synthesizes biogenic polysaccharide-iron hydrogel nanoparticles, known as Fe (III)-exopolysaccharide (Fe-EPS) (Kianpour *et al*., 2016). These can provide important roles in redox capability in biogeochemical cycling, environmental bioremediation and wastewater treatment (Shan-Wei *et al*., 2016).

Finally, it is important to mention that in the phylum of the Bacteroidetes (CFB) is a group that, like other genus mentioned here; is present in different types of habitats such as fresh water, oceans, coral, macroalgae, soils, compost, leaves gymnosperms and angiosperms, dairy products, activated sludge and gastrointestinal tract of animals (*Bacteroides thetaiotaomicron*) where they degrade complex polysaccharides. In marine environments, they have the ability to degrade proteins and complex carbohydrates and recalcitrant sequestered in particulate organic detritus and colonize surfaces. However, in the genus *Flavobacteria*, opportunistic pathogens have been reported that can cause polymicrobial infections (*Bacteroides fragilis*, *B. thetaiotaomicron*, among others) in humans. On the other hand, species of the genus *Flavobacterium* (*F. psychrophilum*, *F. columnare*, *F. branchiophilum*, among others) have caused considerable economic losses in aquaculture farms and wild fish (Thomas *et al*., 2011). In the present work, it was not possible to identify if any of the bacterial species detected in this research have adhered for the manipulation of the fishermen; since the bacterial genera detected in this investigation have been reported in different samples of marine environments. A previous study cited that the indigenous microbiota in the small octopus (*O. variabilis*) could inhibit the colonization of successional species (e.g. *V. vulnificus*) during storage (Lee *et al*., 2017); it will be necessary to carry out more research to verify this theory. However, there is a strong possibility that the identified bacterial flora are part of the native microbial diversity of *O. maya* captured in the Yucatan Peninsula, which can be more analyzed for their biotechnological applications or in other cases, due to their adverse effects on the health of aquatic organisms.

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