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PHA Synthase Genes in Oil-Degrading Marine Bacteria from the Colombian Pacific Coast

Dolly M. Revelo Romo, Marcela Concha Obando, María I. Gómez Perdomo and Pablo Fernández Izquierdo

Universidad de Nariño (UDENAR), Facultad de Ciencias Exactas y Naturales – Departamento de Biología, San Juan de Pasto, Colombia.

***Corresponding author:** Email: margo.revelo@gmail.com, pabfdez@gmail.com, Tel: +57- (2)-7311449

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ABSTRACT:

The phaC genes encode to the PHA synthase enzyme, a key in polyhydroxyalkanoates (PHAs) synthesis. PHAs are biodegradable and biocompatible polymers produced by microorganisms. Some marine bacteria accumulate PHAs using hydrocarbons that are petroleum-derived as a carbon source. In this research 18 oil-degrading marine bacteria isolated from oil-contaminated seawater from the Bay of Tumaco, on the Colombian Pacific coast, were used for detecting the phaC gene and differencing Class I, II (phaC1 y phaC2), and IV PHA synthase. Class I PHA synthase gen fragment was detected in Stenotrophomonas sp. 371B; Class II in Pseudomonas sp. (333(1), 333(2), 380 and 393 strains), Halomonas sp. (394 and 405 strains), Haererehalobacter sp. (338B and 369 strains), and Alcanivorax sp. (400 and 337 strains); Class IV in Bacillus sp. (329A, 329B, 371A, 377C, 353B, 387 and FER1 strains). The bacteria of genera Pseudomonas, Bacillus, Halomonas, Alcanivorax, and Stenotrophomonas are recognized for degrading hydrocarbon and producing PHAs, but few studies on bacteria of genus Haererehalobacter have been proposed; this is the first time they detected the phaC genes in this genus. Therefore, this research shows novel hydrocarbonoclastic marine bacteria with a potential for polymers accumulation and characteristics for future use in bioremediation in the Bay of Tumaco.

Keywords: phaC gene, PHA synthase, oil-degrading marine bacteria, hydrocarbonoclastic marine bacteria, polyhydroxyalkanoates.

[I] INTRODUCTION

A crude oil spill in a marine environment is a pollution problem with negative effects on biotic and abiotic processes [23]. The complex mixture of hydrocarbons enters the marine system from natural oil seeps and natural gas deposits, marine oil transport accidents and deliberate discharges,

and from biomass and biological processes [48]. In a natural marine environment, the amounts of nutrients, especially nitrogen and phosphorus, are insufficient to support the microbial requirements for growth, particularly after a sudden increase in the hydrocarbon level associated with an oil spill [10]. Crude oil pollution constitutes a temporary condition of carbon excess coupled with a limited availability of nitrogen that prompts marine oil-degrading bacteria to accumulate storage compounds [19, 31]. Manilla-Pérez et al. [19] discussed the main storage lipid compounds such as polyhydroxyalkanoates (PHAs), triacylglycerols (TAGs), or wax esters (WEs), accumulated by bacteria under unbalanced growth conditions.

PHAs are intracellular polyesters of hydroxyalkanoates synthesized by bacteria and archaea as carbon and energy storage compounds and have been proposed as substitutes for petroleum-derived polymers and other applications due to their biodegradable and biocompatible properties [11, 18].

The presence of oil-degrading or hydrocarbonoclastic bacteria with metabolic different capacities have been determinate [10], although PHAs accumulation has been occasionally described for some hydrocarbonoclastic marine bacteria [12, 31], such as *Alcanivorax borkunmensis* [32], *Marinobacter hydrocarbonoclasticus*, *A. jadensis* T9, and *Thalassolituus oleivorans* [12].

According to the chain length of the incorporated hydroxyl fatty acids, two types of PHAs are distinguished: scl-PHA (short chain length [C3 to C5], e.g., polyhydroxybutyrate, PHB) and mcl-PHA (medium chain length [C6 to C14], e.g., polyhydroxyoctanoate, PHO) [18]. Copolymers of scl and mcl-PHA, block copolymers of PHA, and homopolymers of PHA are named based on the monomer arrangements in the polymer chains [9]. PHA structure depends of the type of PHA synthase, carbon source, and metabolic routes involved [18]. PHA synthases are key enzymes for PHAs biosynthesis; these enzymes are classified into four classes according to genetic sequence-deduced primary structure, substrate specificity, and subunit composition [26]. The PHA synthase genes and genes for other proteins related to the metabolism of PHA constitute the PHA biosynthesis operons in which different *phaC* genes encode for class PHA synthase: *phaC* (Class I e.g., *Wautersia eutropha*), *phaC1* and *phaC2* (Class II e.g., *Pseudomonas aeruginosa*), *phaC* and *phaE* (Class III e.g., *Allochromatium vinosum*), *phaR* and *phaC* (Class IV e.g., *Bacillus megaterium*).

PHA synthase genes have been described for some marine microorganisms [17, 25], between which stand the oil-degrading bacteria *A. borkumensis* strain SK2, (*phaC1* and *phaC2*) [31]. Detecting *phaC* genes in bacteria has proven to be an instrument for the precise and directed selection of bacteria from different genera, having the potential for accumulating PHAs [28, 34, 35, 39]. We have conducted a literature review and so far there are no reports on the detection of the *phaC* genes in hydrocarbonoclastic marine bacteria as a screening method of PHAs accumulation. The *phaC* gene detection informs the capacity to express the PHA synthase enzyme, and the conditions enabling PHA production are determinate using another approach [16].

The Bay of Tumaco (Nariño) on the Colombian Pacific Coast presents oil spill areas [6, 41] and stands among the most polluted water in the world. In previous researches we demonstrated the presence of novel genera of hydrocarbonoclastic marine bacteria in this zone [30]; therefore, the aim of this study was to detect and differentiate the *phaC* genes encoding PHA synthase enzyme in bacteria isolated from seawater contaminated with oil crude. This research was directed to find novel marine hydrocarbons-degrading bacterial strains with future uses to express the PHA synthase enzyme and bioremediation applications.

[II] MATERIALS AND METHODS

2.1. Bacterial strains and growth conditions

Bacterial strains used: *Pseudomonas aeruginosa* BUN010 (*phaC* positive) and *Escherichia coli* BUN001 (*phaC* negative), a collection of microorganisms from the Universidad de Nariño. Nutritive agar medium (OXOID) was used to maintain the bacteria and the plates were incubated for 24 hours at 25˚C.

Oil-degrading marine bacteria from oil contaminated seawater were obtained in Upstream Flow Bioreactors (UFB) according to protocol described by Revelo *et al.* [30]. The following eleven isolated bacteria included in this study were previously characterized by partial sequencing of the 16S rRNA gene [30]: *Pseudomonas* sp. strains 333(1), 333(2), and 380; *Bacillus* sp. strains 329A, 329B, 371A, 377C, and FER1; *Halomonas* sp. strains 394 and 405; and the strain *Haererehalobacter* sp. 338B.

Seven new bacterial isolates were selected by their ability to degrade hydrocarbons and were cultured using the medium described by Brito *et al.* [4]: modified with n-octane as the sole carbon and energy source. Bacterial strains were maintained as described by Revelo *et al.* [30]. The plates were maintained in an aerobic condition at 25˚C for 24 hrs.

2.2. Partial characterization of the 16S ribosomal gene (16S rRNA gene)

With the protocol described by Revelo *et al.* [30], seven new bacteria were characterized in this study. A phylogenetic tree of all 18 hydrocarbonoclastic marine bacterial strain similarity was based on analyzing 16S rRNA gene partial sequences and was constructed using the software MEGA 5 [40] from the 603 to 777 bp 16S rRNA gene sequences by the Neighbour-Joining algorithm [33] using the P distance as a parameter, and was submitted to Felsentein's Bootstrap test.

Sequence accession numbers: The eighteen 16S rRNA gene partial sequences have been deposited in GenBank under accession numbers: JN383421 - JN383425, JN661559 - JN661567, JQ653247 - JQ653250.

2.3. Determining *phaC* genes in oil-degrading marine bacteria

DNA extractions and the amplification of 551 (G-D and G-1R primers) and 491 (G-D and G-2R primers) bp's fragment of the gene encoding PHA synthase were carried out using a protocol proposed by Revelo *et al.* [28], which uses conventional PCR and semi-nested PCR. A total of 2,5 µL of DNA bacterial lysate were added as a template in conventional PCR, and 2 uL DNA template from the first *phaC* gene amplified product were added to this reaction from positive products and 5 uL for negative products.

Conventional and semi-nested PCR products were sent to the MACROGEN Company, Korea, for sequencing service. The search for similarity amongst *phaC* gene partial sequence from bacterial strains was done through alignment with the available sequences in the public nucleotide databases at the National Center for Biotechnology Information (NCBI) by using its World Wide Web site (http://www.ncbi.nlm.nih.gov) and the BLAST algorithm [3].

Sequence accession numbers: The *phaC* gene partial sequences of the 5 bacterial strains obtained with the conventional and semi-nested PCRs have been deposited in GenBank under accession numbers: JQ653251 - JQ653255.

2.4. Differentiating Class II *phaC1* and *phaC2* genes and Class IV *phaC* genes in oil-degrading marine bacteria

DNA extractions and the amplification of 1965 (E1-D and E1-R primers) and 2335 (E2-D and E2-R primers) bp's fragment of the Class II *phaC1* and *phaC2* genes respectively, were carried out using a protocol proposed by Revelo *et al.* [28].

DNA extractions and the amplification of 540 (FDM and RDM2 primers) bp's fragment of the gene encoding Class IV PHA synthase was

carried out using a protocol proposed by Revelo *et al.* [29].

All PCR reactions were performed in a Multigen Gradient (LABNET) and Techne (TECHGENE) temocyclers following the conditions in reference to Revelo *et al.* [28, 29]. PCR products were conserved at -20 °C. Electrophoresis on 1% (w/v) agarose gels (1X-TBE) was used for detecting PCR amplification products. λ DNA/*Hind* III (Fermentas) and HyperLadder™ IV (Bioline), were used as DNA size markers. Run conditions were proposed by Revelo et al. [28]. The gels were stained with EZ-Vision Three™ (AMRESCO) and amplified DNA fragments were visualized under DigDoc-Lt System-Benchtop 3UV™ digitaliser.

Sequence accession numbers: Some PCR products were sent to MACROGEN for sequencing. Ten partial sequences of *phaC* genes obtained from fragments amplified with specific primers have been deposited in GenBank under accession numbers: JQ653256 - JQ653265.

[III] RESULTS

3.1. Partial 16S rRNA gene sequence analysis in hydrocarbonoclastic marine bacteria

All 18 bacterial isolates were selected from the UFBs [30] and were capable of n-octane-degrading. Seven bacteria were characterized in this research and were closely related as: 393 with *Pseudomonas* sp. (99% similarity), 353B with *B. subtilis* (99% similarity), 387 with either *Bacillus firmus* or *B. oceanisediminis* (100% similarity), 396 with *Haererehalobacter* sp. (99% similarity), 337 with *Alcanivorax* sp. (99% similarity), 400 with *A. dieselolei* (100% similarity), and 371B with *Stenotrophomonas* sp. (99% similarity). Therefore, the bacterial isolates 393 were classified as *Pseudomonas* sp.; 353B and 387 as *Bacillus* sp.; 396 as *Haererehalobacter* sp.; 400 and 337 as *Alcanivorax* sp.; and 371B as *Stenotrophomonas* sp.

The 18 hydrocarbonoclastic marine bacteria included here were grouped into 2 clusters corresponding to Proteobacteria and Firmicutes phylum (Figure 1). The phylogenetic tree shows five clusters. Four clusters belong to the Proteobacteria phylum (gamma-Proteobacterias): the first cluster corresponds to bacteria from the *Pseudomonas* genus, the second cluster is formed by a bacterial species from the *Halomonas* and *Haererehalobacter* genera, the third cluster corresponds to bacteria from the *Alcanivorax* genus, and *Stenotrophomonas* sp. 371B was in the fourth cluster, similar to *S. maltophilia.* The fifth cluster belongs to the Firmicutes phylum, formed by bacterial species from the *Bacillus* genus.

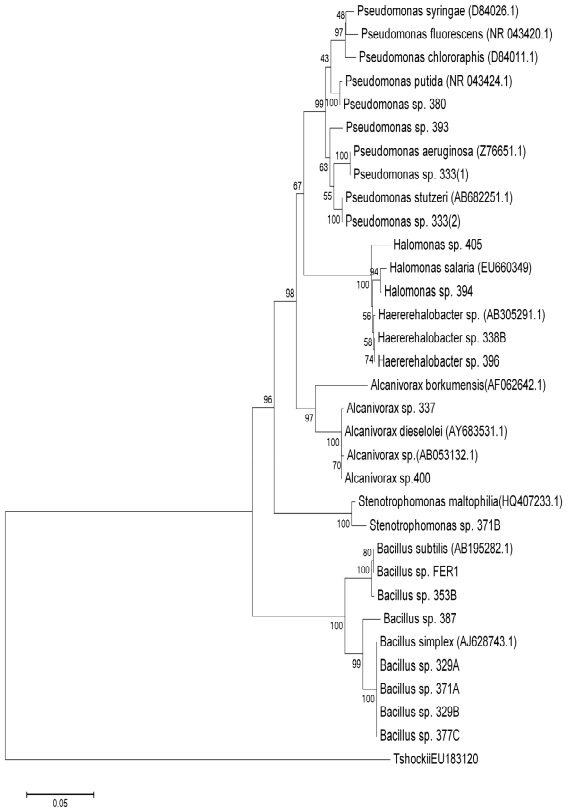

Fig: 1. Phylogenetic tree of hydrocarbonoclastic marine bacterial strain similarity basedon analysing 16S ribosomal gene partial sequences. External group, *Thermogladius shockii*.

3.2. Amplifying the *phaC* gene fragment

The 18 bacterial strains had the *phaC* gene fragment in their genotype. The *phaC* gene fragment was detected for conventional PCR in *Halomonas* sp. 394 and *Pseudomonas* sp. 333(1); however, all bacteria strains could be detected when using semi-nested PCR confirmed *phaC* gene presence (Figure 2). Some sequences obtained were similar to sequences of the *phaC* gene in GenBank, which established the presence of the *phaC* gene in hydrocarbonoclastic marine bacteria obtained in this study.

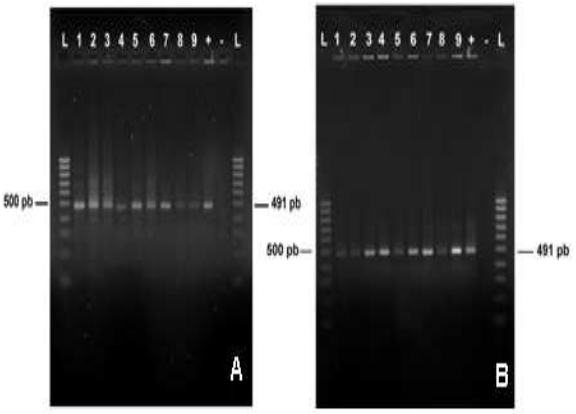

Figure 2. Confirmation by semi-nested PCR. A) Lanes: L, molecular weight marker (HyperLadder™ IV); 1, *Bacillus* sp. 329A; 2, *Bacillus* sp. 329B; 3, *Pseudomonas* sp. 333(1); 4, *Pseudomonas* sp. 333(2); 5, *Alcanivorax* sp. 337; 6, *Haererehalobacter* sp. 338B; 7, *Bacillus* sp. 353B; 8, *Bacillus* sp. 371A; 9, *Stenotrophomonas* sp. 371B; (+), *P. aeruginosa* BUN010 (*phaC*+); (-), *E. coli* BUN001 (negative control). B) Lanes: L, molecular weight marker (HyperLadder™ IV); 1, *Bacillus* sp. 377C; 2, *Pseudomonas* sp. 380; 3, *Bacillus* sp. 387; 4, *Pseudomonas* sp. 393; 5, *Halomonas* sp. 394; 6, *Haererehalobacter* sp. 396; 7, *Alcanivorax* sp. 400; 8, *Halomonas* sp. 405; 9, *Bacillus* sp. FER1; (+), *P. aeruginosa* BUN010 (*phaC*+); (-), *E. coli* BUN001 (negative control).

3.3. Differentiating Class II and Class IV PHA synthase organization in oil-degrading marine bacteria

Ten out of eighteen bacterial strains were present in the Class II PHA synthase operon organization: *Pseudomonas* sp. 380, *Pseudomonas* sp. 393, *Haererehalobacter* sp. 396, and *Haererehalobacter* sp. 338B amplified both *phaC1* and *phaC2* genes, but *Halomonas* sp. 405 and *Halomonas* sp. 394 only amplified the *phaC1* gene, and *Pseudomonas* sp. 333(1), *Pseudomonas* sp. 333(2), *Alcanivorax* sp. 337, and *Alcanivorax* sp. 400 only amplified the *phaC2* gene (results not shown). Seven bacterial strains were present in the Class IV PHA syntase: *Bacillus* sp. 329A, *Bacillus* sp. 329B, *Bacillus* sp. 353B, *Bacillus* sp. 371A, *Bacillus* sp. 377C, *Bacillus* sp. 387, and *Bacillus* sp. FER1 (results not shown). *Stenotrophomonas* sp. 317B could not amplify the *phaC1*, *phaC2* (Class II), or *phaC* (Class IV) genes; however, it did amplify the *phaC* gene using semi-nested PCR, concluding that this bacteria was present in Class I PHA synthase.

These results were confirmed with ten of the fragments amplified by specific primers on *phaC* genes, which presented sequences that aligned with sequences of *phaC* genes in the databases.

[IV] DISCUSSION

The knowledge level bacterial genus and species is important due to it is possible to learn about nutritional requirements and other factors by growing and handling the bacteria *in vitro*. This research characterized seven oil-degrading marine bacteria corresponding to: *Pseudomonas* sp.393, *Bacillus* sp. (353B and 387 strains), *Haererehalobacter* sp. 396, *Alcanivorax* sp. (400 and 337 strains), and *Stenotrophomonas* sp. 371B. According to literature the bacteria with the ability to degrade hydrocarbons in marine environments belong to several genera such as *Acinetobacter, Aeromonas, Bacillus, Burkholderia, Halomonas, Kocuria, Paracoccus, Pseudomonas, Rhodobacter, Rhodococcus, Staphylococcus, Vibrio* [23], *Alcanivorax, Marinobacter,* and *Cycloclasticus* [48]. There are many reports about bacterial species belonging to genera *Pseudomonas* [2, 44], *Bacillus* [2, 8, 23],

and *Alcanivorax*, grouping the obligate hydrocarbonoclastic marine bacteria (OHCB); this exhibits a narrow substrate spectrum (obligate hydrocarbon utilization) and over the past few years has been shown to play an important role in the biological removal of petroleum hydrocarbons from contaminated sites [12, 19, 20 48]. *Stenotrophomonas* genus is well known as polycyclic aromatic hydrocarbons (PAHs)-degrading [22, 47]. Regarding *Haererehalobacter*, there are few studies on this genus so far in the literature review [46].

The distribution of marine oil degrading bacteria was agreed upon and recognized by Kersters *et al.* [13]. All bacterial strains of *Halomonas*, *Pseudomonas*, *Alcanivorax*, and *Haererehalobacter* genera obtained in this research were grouped into 4 clusters belonging to Proteobacteria phylum (Gamma-proteobacteria class), which is now the largest and most diverse phylogenetic lineage. It is important to note that strains of *Halomonas* and *Haererehalobacter* form a cluster with bootstrap presented 100% confidence values. Therefore, other tests are required for their taxonomic classification. The *Bacillus* genus bacteria obtained here belong to the Firmicutes phylum and future studies are required to stabilise the possible intraspecific diversity.

The above results can be compared with those obtained by Velmuruga *et al.* [45], who examined 136 marine bacterial isolates, of which 5 were identified as novel members and the 131 remaining isolates were grouped in 5 major lineages of the phylum Firmicutes, alpha-proteobacteria, gamma-proteobacteria, High G + C, and Bacteroides. It is important to note that in this bacterial community of marine sediments, the gamma-proteobacteria were highly divergent and were represented by the genera: *Vibrio, Marinobacterium, Photobacterium, Pseudoalteromonas, Oceanisphaera, Halomonas, Alteromonas, Stenotrophomonas,* and *Pseudomonas*. It was concluded that 3.6% were novel isolates. In our study, of 18 bacterial strains, 11.1% accounted for bacteria belonging to the *Haererehalobacter* genus and 88.9% were represented by bacteria belonging to the genera widely recognized as hydrocarbonoclastic bacteria.

The bioreactors used in our previous research [30] simulated a marine environment with unbalanced condition nutrients provided by the presence of an excess carbon source (crude oil) to respect the nitrogen source. Therefore, the bioreactors presented proper unbalanced conditions for PHA accumulation by bacteria. In this research we detected the *phaC* gene in the 18 bacterial strains. Based on the reports concerning the characterization of the *phaC* gene in marine bacteria [25 17, 28, 31], our research represented a significant contribution to knowledge about the presence of this gene in hydrocarbon-degrading marine bacteria. This research showed the potential of these 18 bacterial strains because they possess the PHA biosynthesis operon. These *phaC* positive bacteria are bio-factories that degrade hydrocarbons and are potential PHAs accumulators because they have the capacity to express the PHA synthase enzyme. It was noted that the degenerate character of the primers allowed the detection of the *phaC* gene fragment, including bacteria genera not considered by Revelo *et al.* [28] in the design of the primers, such as *Bacillus*, *Halomonas*, *Haererehalobacter*, and *Alcanivorax*.

phaC genes to Stenotrophomonas. The oil-degrading bacterial strain *Stenotrophomonas* sp. 371B, closely related to *S. maltophilia*, contains the *phaC* gene encoding Class I PHA synthase. This is an interesting result because it had recently been reported that the *phaC* genes are present in bacteria of the genus *Sthenotrophomonas.* In this way Crossman *et al.* [7] studied a PHA synthase in *S. maltophilia* K279, and Singh and Parmar [38] reported that in the *S. maltophilia* genome the *phaA/B/C/Z* genes were related to PHB production. This operon

organization is according to the Class I PHAs biosynthesis operon described in Rehm [26].

phaC genes to Pseudomonas. In our research we obtained the *phaC* positive bacterial strains *Pseudomonas* sp. 333(1), 333(2), 380, and 393. Therefore, they can be PHAs accumulating. It is widely recognized that the bacteria of *Pseudomonas* genus are mcl-PHAs producing [43]. In the present work we manage to detect the genes of a Class II PHA synthase system in all pseudomonds characterized here. Out of sequence to *phaC* gene fragment detecting, *Pseudomonas* sp. 333(1) was similar to sequences of Class II PHA synthase. This system has been cloned and extensively characterized in species of this genus, including *PhaC1* and *phaC2* [1, 27].

phaC genes to Halomonas. The oil-degrading marine bacterial strains *Halomonas* sp. 394 and 405 obtained in this research have the *phaC* gene; the result of this is an important report about PHA biosynthesis genes in the *Halomonas* species and constitutes them as potential candidates to produce PHAs. Besides this, in both bacteria the *phaC1* gene of Class II PHA synthase operon was detected, as well as the sequence of *phaC* gene fragment of *Halomonas* sp. 405, which is similar to Class II PHA synthase. This is an important contribution to the little information available on genomics and genes related to PHA biosynthesis from halofilics bacteria existent today [5]. The authors show that *phaC1, phaZ,* and *phaC2* genes and others genes related to PHA metabolism to strain *Halomonas* sp. TD01, and PHA synthases enzymes characterized, are novel, especially the encoding by *phaC1*. The sequence of this protein are similar to Class I PHA synthase and were found to be PHB producing.

Other studies show the scl-PHAs producing [24] and the copolymers [14]. *Halomonas* species are able to co-produce PHA and osmolytes [25]. Marine bacteria of *Halomonas* genus that have been studied for their ability to accumulate PHAs include *H. profundus* sp. nov. [37] and *H. hydrothermalis* [36].

phaC genes to Haererehalobacter. *Haererehalobacter* sp. 338B and 396 have the *phaC* gene. There are few studies on this genus so far in the literature review [46], and there are no known reports about *Haererehalobacter* genus bacteria as PHAs accumulators or PHAs biosynthesis genes. Therefore, this research can be the first report of *phaC* positive hydrocarbonoclastic marine bacteria belonging to the *Haererehalobacter* genus. In our research is a relevant result detecting *phaC1* and *phaC2* genes of Class II PHA syntase to strains *Haererehalobacter* sp. 338B and *Haererehalobacter* sp. 396.

phaC genes to Acanivorax. In our research we obtained two OHCB: *Alcanivorax* sp. 337 and *A. dieselolei* 400, which have the *phaC* gene and thus are potential PHAs accumulators. Both *phaC1* and *phaC2* genes detected *Alcanivorax* sp. 337 and *Alcanivorax* sp. 400. In recent researches Kalscheuer *et al.* [12] showed that the genome of *A. borkumensis* has these two genes that express differentially. Sabirova *et al.* [31] reported that the strain *A. borkumensis* SK2 has the enzymes in PHA production under conditions of carbon excess. Out of the genes of PHA synthase, the *phaC* (ABO_1418) gene expresses only the bacteria cultivated on alkane; the other *phaC* (ABO_2214) gene is not expressed in detectable level; but is PHA producing under high C/N relations in cells that grown on substrates whit out alkanes. Sabirova *et al.* [32] demonstrated that *A. borkumensis* SK2 clearly has the genetic equipment to synthesize PHAs and showed the hyperproduction of extracellularly deposited PHA in a mutant strain.

In conclusion the strains belong to genera as *Pseudomonas, Alcanivorax, Halomonas* and *Haererehalobacter* have Class II PHA synthase. The three last genera are novel because is yet recent the research about PHA biosynthesis operons, the enzymatic activity of Class II PHA

synthase and the use of carbon resources as oil's hydrocarbons.

phaC genes to Bacillus. The oil-degrading marine bacteria belong *Bacillus* genera: *Bacillus* sp. 329A, 329B, 371A and 377C (similarity to *B. simplex*) and *Bacillus* sp. 353 and FER1 (similarity to *B. subtilis*), and *Bacillus* sp. 387, were *phaC* positive and constituted as potential PHAs producers. Besides these genes belong to the Class IV PHA synthase. Regarding *phaC* genes in *Bacillus* genus there are main research including to McCool and Cannon, [21], which characterized in *B. megaterium* of Class IV PHA synthase whit two subunits (PhaC and PhaR) and this is related to scl-PHAs synthesis [26]. *Bacillus* has been recognized for its expression of PHA synthesis genes and recent efforts have shown its potential to generate PHAs [15]. Marine bacteria of *Bacillus* genus have been reported as PHB accumulator, however, few studies that have been reported about the ability of PHAs accumulation in *B. simplex* [42].

[V] CONCLUSION

The sequencing of 16S rRNA gene fragments allowed the characterization of 7 marine bacterial isolates as belonging to recognized genera as hydrocarbon degraders: *Pseudomonas*, *Bacillus*, *Stenotrophomonas*, *Alcanivorax*, and *Haererehalobacter*. The latter genus had not been reported for marine bacteria hydrocarbonoclastic *phaC* positive. Therefore, this result constitutes an important report of the marine bacteria hydrocarbonoclastic of the Bay of Tumaco (Nariño).

The *phaC* gene detection in these bacteria proposed them as candidates with enzymatic potential for PHAs accumulation; they could also be used as cheap carbon sources in environments contaminated with hydrocarbons petroleum and thus contribute to the bioremediation process, as evidenced by reports about the functions and metabolic requirements of the genera presented here. From this viewpoint clean-up marine oil spills and/or future production of biodegradable plastics might be carried out at the same time.

Besides the use of marine water as a base medium for growing bacteria [30], this also decreases the costs for PHA producing and providing the basic conditions for development of marine bacteria.

It was determined that 18 bacterial strains were *phaC* positive and that the bacteria belong to genera *Alcanivorax, Halomonas, Pseudomonas* and *Haererehalobacter,* present Class II PHA synthases; the bacteria of the genus *Bacillus* presents in Class IV PHA synthase; and *Stenotrophomonas sp.* 371B. presents in Class I PHA synthase.

Haererehalobacter sp. 338B and *Haererehalobacter* sp. 396 are reported as hydrocarbonoclastic bacteria *phaC* positive for the first time.

FINANCIAL DISCLOSURE

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