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Determination of bacteria with potential for total hydrocarbon biodegradation at El Waffe Dock in Turbo, Antioquia, and analysis of environmental policies aimed at the bioremediation of coastal areas

Determinación de bacterias con potencial biodegradador de hidrocarburos totales en muelle El Waffe de Turbo, Antioquia y análisis de las políticas ambientales encaminadas a la biorremediación de zonas costeras

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Abstract

Petroleum-derived hydrocarbons are chemical structures that are contaminants, toxic, mutagenic, and carcinogenic, affecting the trophic chain of ecosystems. These compounds are primarily generated through anthropogenic activities, and their discharges into marine-coastal areas are mainly due to industrial and maritime transportation sectors. In this study, a bioremediation technique involving the bioprospecting of bacteria with the capacity to degrade total hydrocarbons was used. Subsequently, selective pressure was applied under conditions of a sole carbon source, successfully isolating 11 morphotypes in pure culture media supplemented with hydrocarbons, thereby demonstrating the presence of microorganisms with biodegradative potential. Three sampling points along El Waffe Dock in Turbo, Antioquia, Colombia were analyzed in a zigzag pattern. Additionally, the microscopic and macroscopic characteristics of the isolated strains were compared with related literature to approximate the identification of the microorganism. Finally, a review of the political and regulatory frameworks surrounding hydrocarbon bioremediation and the generation of new knowledge in this field in Colombia was conducted.

Keywords: bioremediation, coastal zones, environmental policies, hydrocarbons

Resumen

Los hidrocarburos derivados del petróleo son estructuras químicas contaminantes, tóxicas, mutagénicas y cancerígenas que afectan la cadena trófica del ecosistema; estos compuestos son generados, principalmente, a partir de actividades antropogénicas y cuyos vertimientos en zonas marino-costeras, son derivadas por los sectores económicos industriales y de transporte marítimo. Para el presente trabajo, se utilizó una técnica de biorremediación consistente en la bioprospección de bacterias con capacidad biodegradadora de hidrocarburos totales; luego, se generó una presión selectiva en condiciones de única fuente de carbono, logrando el exitoso aislamiento de 11 morfotipos en medios de cultivos puros suplementados con hidrocarburos, demostrándose la presencia de microorganismos con potencial biodegradador. En este trabajo,

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fueron analizados tres puntos de muestreo en forma de zigzag a lo largo del muelle El Waffe de Turbo, Antioquia, Colombia. Adicionalmente, se realizó una comparación de las características microscópicas y macroscópicas de las cepas aisladas con literatura relacionada para acercarse a una identificación del microorganismo. Finalmente, se efectuó una revisión de los ámbitos políticos o normativos que rodean la biorremediación de hidrocarburos y la generación de nuevos conocimientos en esta materia en Colombia.

Palabras clave: biorremediación, hidrocarburos, políticas ambientales, zonas costeras

INTRODUCTION

Hydrocarbons are compounds derived from petroleum and classified as natural resources. They are widely used globally due to their importance in various industrial sectors. Generally, hydrocarbons undergo transformations to support the transportation industry, energy production, or the manufacture of plastic materials. Therefore, hydrocarbons are a fundamental pillar of the global economy, including that of Colombia (Marín, 2014).
The chemical structure of hydrocarbons allows them to exist in both saturated and unsaturated states, with their bonds and other ringed structures determining their level of reactivity or instability upon contact with the environment. The most common and toxic compounds in crude oil or petroleum are benzene, toluene, ethylbenzene, and xylene (Kotoky et al., 2022; Quiceno-Pérez & Ríos-Osorio, 2014; Rodríguez-Trigo et al., 2007). Consequently, the presence of these compounds in oceanic and coastal ecosystems due to ship accidents or indiscriminate use affects all organisms in the trophic chain, starting with phytoplankton and zooplankton. Fish larvae, benthos, and crustacean populations in estuaries and reefs are particularly diminished by their presence (Buskey et al., 2016; Pérez-Hernández et al., 2020).

Polluted ecosystems have an intrinsic capacity to degrade quantities of organic substances, a process known as natural attenuation. This process is primarily linked to oxidative reactions facilitated by the metabolic activities of microorganisms, which can reduce pollutant concentrations (Gómez-Mellado et al., 2020).

Despite the commitment and initiatives of governments worldwide to explore and invest in maritime safety and environmental protection measures to counteract the excessive use of fuels and other petroleum products, such as the creation of the IMO (International Maritime Organization), a large volume of hydrocarbons continues to be released. Historically reported accidents have exceeded three million barrels spilled, amounting to 27,000 tons of oil, into marine waters between 2020 and 2023 (Hernández-Ruiz et al., 2021; ITOPF, 2024).

In Colombia, the National Authority of Environmental Licenses (ANLA), the entity responsible for endorsing intervention studies and compiling the effects of hydrocarbon-related accidents in the country, reports that 14,656,983.64 barrels of hydrocarbons have been spilled in the Department of Antioquia over the last 22 years.

Additionally, in the Gulf of Urabá, there have been fuel spills that are not recorded in the ANLA database (Cruz et al., 2022). Despite this, the spill with the greatest impact in recent years, reported by local media, was the spill of 1000 to 1300 gallons (equivalent to between 23.81 and 30.95 barrels) of hydrocarbons in April 2013 after a collision between a ship and a barge in the maritime jurisdiction of Turbo, Antioquia (El Universal, 2013; Minuto 30, 2010; Red de Desarrollo Sostenible, 2013).

The El Waffe dock in the District of Turbo, Antioquia, has been a source of economic resources for the surrounding community for many years (Alcaldía Distrital de Turbo, 2020). Although not considered a port, the El Waffe dock traditionally allows daily traffic of passengers and goods, incoming and outgoing from various areas of the Caribbean and Colombian Pacific. This activity has caused alterations in the ecosystem as a result of spills (Salas-Tovar & Murillo-Hinestroza, 2013). The district's governmental entities have undertaken actions for the urban renewal of this pier and its surroundings to improve mobility, the quality of life of the inhabitants, and the positive perspective of travelers (Alcaldía Distrital de Turbo, 2020). However, in Colombia, there is limited research on bioremediation in marine-coastal environments, with most focusing on the northern part of the Colombian Caribbean coast, particularly in the Bay of Cartagena (Echeverri-Jaramillo et al., 2010).

Some of the known strategies for hydrocarbon removal, as reported by different authors such as Gómez-Mellado et al. (2020) and Quiceno-Pérez & Ríos-Osorio (2014), have been carried out through physical methods such as adsorption by activated carbon or chemical methods such as solvent extraction and chemical oxidation. Biological methods, although less popular, have been applied using aerobic and anaerobic microorganisms (Ma et al., 2011; Orozco-Zárate, 2021). In remediation strategies, different techniques can be used separately or in combination to achieve higher pollutant removal efficiency.Among the various physical, chemical, and biological techniques, biodegradation by microbial agents such as bacteria or fungi is currently considered the least expensive alternative for transforming contaminants (Barrios-San Martín, 2011). A wide variety of microorganisms capable of using hydrocarbons as their sole carbon source have been reported, including *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* (Muthukumar et al., 2023), *Rhodococcus* spp. (Chirre-Flores et al., 2019), *Bacillus pumilus*, *Serratia marcescens* (Pardo-Castro et al., 2004), *Klebsiella* spp. (Rabelo-Florez & Márquez-Gómez, 2020), and *Enterobacter* spp. (Haritash & Kaushik, 2009).

The IMO has published intervention guidelines for potential oil spills, although organizations and/or state institutions have the authority to decide which methodologies are used to partially or completely remediate contamination in natural environments. Therefore, it is necessary to analyze the relationship between Colombia's environmental policies to identify possible routes for the development of new research projects that can strengthen technical knowledge and the application of bioremediation in areas affected by oil spills in national territories. This aligns with the goals established by the Convention on Biological Diversity and the National Council for Economic and Social Policy (CONPES) - Colombia Sustainable Bioceanic Power. These organizations recognize the lack of databases, studies, and usage of regions associated with and near the Panama Canal and the coastal areas of the Pacific and Colombian Caribbean. Similarly, marine and estuarine environments are recognized as fundamental habitats for the development of a variety of species, proposing multiple strategies and commitments for their restoration and sustainable use (United Nations [UN], 1992; Orozco-Zárate, 2021).

Although microorganisms with hydrocarbon biodegrading potential and policies related to the use of bioremediation techniques in contaminated coastal areas have been described in the literature, the District of Turbo lacks studies or projects that confirm their presence and mitigate their impact on hydrocarbon-contaminated sites. Consequently, the objective of this research is to determine the biodegradation potential of hydrocarbons by cultivable bacteria present in the waters of El Waffe dock in Turbo, Antioquia, and to analyze national environmental policies aimed at bioremediation in coastal areas.

MATERIALS AND METHODS

Sampling process

Three sampling points were selected for marine sediment collection in the Waffe area of the Turbo District, Antioquia, following the guidelines provided in the *Manual of Analytical Techniques for the Determination of Physicochemical Parameters and Marine Pollutants* (INVEMAR, 2003). The points were spaced approximately 100 meters apart in a zigzag pattern (Figure 1). It is important to note that sampling was conducted only once, during the morning hours and at low tide.

A Van Veen SG dredge was used to collect marine sediment, with three launches made to obtain different portions of the sample, which were then composited. The sample was stored in sterile resealable bags, leaving a layer of air inside.

At each of the sampling points, the water transparency parameter was determined in triplicate using a *Secchi* disk. Based on the reference method SM 2550 B (APHA, 2012), the ambient temperature was measured in triplicate with a mercury thermometer to obtain a time average of this variable. Additionally, seawater samples were taken 20 cm from the surface, and pH was determined in situ (HATCH®). For sediment

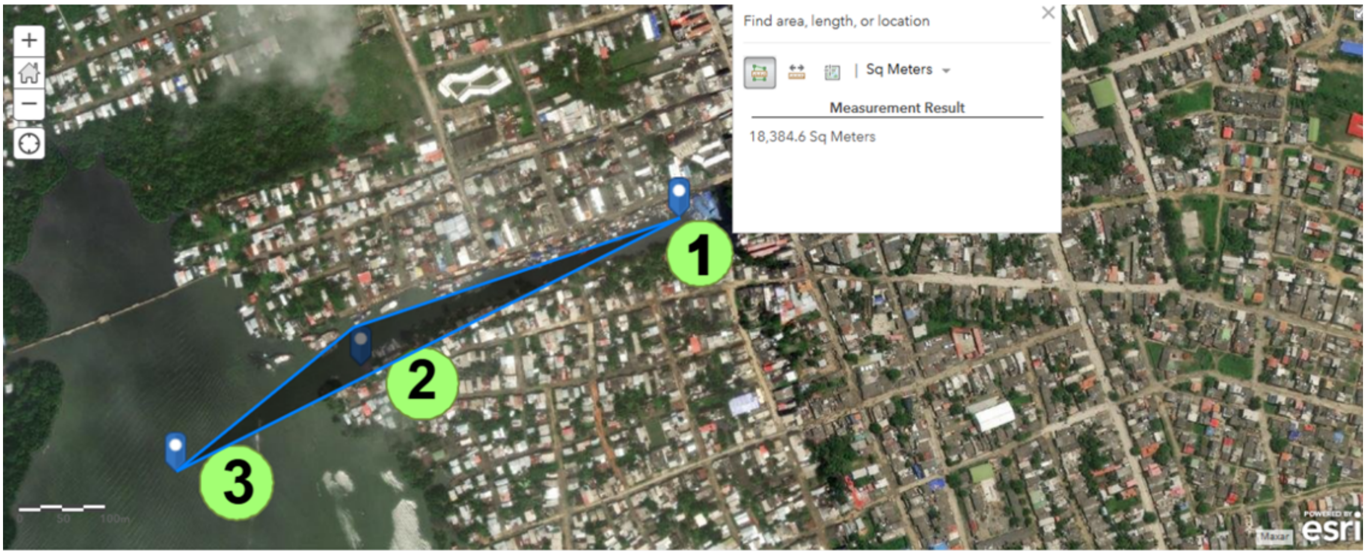

Figure 1. Map of geographic coordinates of the three sampling sites: 8°05'27.7"N 76°43'45.7"W; 8°05'22.4"N 76°43'57.7"W; 8°05'18.2"N 76°44'04.7"W.

samples, pH was determined in the laboratory following the reference method SM 4500 H+B (APHA, 2012). All samples were transported at temperatures between 4-8 °C to the Universidad de Antioquia laboratory in Apartadó, where they were immediately processed.

Selective pressure

To isolate bacteria capable of degrading hydrocarbon-derived compounds, a selective pressure test was conducted, adapting the methodology from the protocol of the *Institute of Marine and Coastal Research-INVEMAR* titled "Native Marine Bacteria Degrading Persistent Organic Compounds in Colombia" (Gómez et al., 2006). Sediment samples from each point were analyzed in triplicate, with an initial enrichment made by combining 450 mL of collected seawater and 50 g of the sample. Five milliliters of the decanted samples were then transferred to 45 mL of nutrient broth and incubated at 150 rpm and 28 ºC for seven days. Subsequently, 5 mL of the sample was transferred to 45 mL of minimal medium (2.4 g/mL NaCl, 0.07 g/mL KCl, 0.1 g/mL NH4NO3, 0.2 g/mL KH2PO4, 0.3 g/mL Na2HPO4, 0.1 g/mL MgSO4 in 1000 mL of H2O) supplemented with 1% hydrocarbons, consisting of a 2.4% v/v mixture of motor oil in regular gasoline, this being the only carbon source and giving the medium a reddish color. The samples were incubated again for 7 days at 150 rpm and 28 °C. This step was repeated once more.

The treatments named T1, T2, and T3 correspond to the potential microorganisms isolated from sampling points 1, 2, and 3, respectively. Additionally, controls were used to compare the results: T4 as a negative control with sterile distilled water, T5 as a control with unsterilized seawater, T6 as a positive control with *Pseudomonas aeruginosa*, T7 as a negative control with minimal saline mineral medium, and T8 as a negative control with minimal saline mineral medium + 1% hydrocarbon.

Isolation of colonies in axenic medium

Surface seeding was performed from the samples, and morphotypes with distinct macroscopic characteristics were isolated on nutrient agar to obtain axenic cultures and characterize them. Each isolate underwent Gram staining, macroscopic description of the colony with respect to shape, edge, surface, texture, and elevation, as well as microscopic examination. Additionally, a literature review was conducted on reports of hydrocarbon biodegradation in coastal areas to identify the respective bacterial genera isolated and to facilitate possible identification by comparison.

Simultaneously, a bibliographic search focused on studies or research conducted between 2010 and 2023, specifically on bioprospecting in marine-coastal zones, was carried out. The aim was to identify communities with potential for hydrocarbon biodegradation. The following bibliographic databases and search engines were used: ScienceDirect, Scielo, Redalyc, PubMed, EBSCO, and Scopus. During the search process, keywords and combinations thereof were defined to maximize the number of related publications, such as bioremediation, hydrocarbons, sediments in Colombia, and biodegradation. These keywords were applied in searches within databases and indexed journals, selecting original articles, theses, and abstracts that provided data and studies related to microbial degradation techniques, phytoremediation, enzymatic degradation, *in situ* bioremediation, *ex situ* bioremediation, and sediment bioremediation. Records that did not provide relevant information on hydrocarbon bioremediation or did not meet the conditions and parameters similar to those of this research, such as reviews and articles on soil bioremediation, were excluded.

Regulatory review

Additionally, a search was conducted for government regulations or policies, as well as government plans that involve or relate to bioremediation, biodegradation, or studies on biological biodiversity focused on hydrocarbons within Colombian territory, specifically segmented to the District of Turbo, Antioquia.

The gathered information was then organized and characterized from the national to the district level, following a tiered legal structure based on the Kelsenian Pyramid (Galindo-Soza, 2018).

RESULTS AND DISCUSSION

Sampling

The physical parameters obtained at the three sampling points (Table 1) were compared with the pH and Dissolved Oxygen (DO) values from the ICAM (Maritime Water Quality Indicator). The results suggest that the estuarine waters in the analyzed area, which showed pH values of 7 and DO values below 5 mg/L, are inadequate for the development of marine flora and fauna (Vivas-Aguas & Navarrete-Ramírez, 2014). This indicates that the ecosystem may be adversely affected by pollutants present in the sampled area, potentially stemming from anthropogenic activities in the coastal zone, whether from domestic or industrial sources. These findings are consistent with the conclusions of Salas-Tovar and Murillo-Hinestroza (2013) and Murillo-Hinestroza et al. (2017), whose analysis of the physicochemical quality parameters of Turbo Bay demonstrated that the pollutants contributed by the pier significantly increase the pollution load in the bay (Salas-Tovar & Murillo-Hinestroza, 2013).

The data suggest that, in the El Waffe area, the persistence of waters with low DO does not favor the development of biological communities. This situation could be attributed to the presence of a large amount of organic material in the water, the constant deposition of solid waste from anthropogenic activities, and the lentic characteristics of the water (Murillo-Hinestroza et al., 2017). The impacts on the ecosystem are also reflected in studies of physicochemical parameters conducted in the areas near the El Waffe dock (Table 2). These studies reveal changes when comparing high and low tide conditions. Notably, this lentic characteristic of the ecosystem has resulted in DO values falling below 3 mg/L (Salas-Tovar & Murillo-Hinestroza, 2013). Additionally, these studies report variations in DO, conductivity, and dissolved solids under different tidal conditions, indicating fluctuations in water quality. These results underscore the importance of addressing environmental impacts in the area and highlight the need for implementing measures to manage and conserve the marine-coastal ecosystem.

Table 1. Results of sampling points 1, 2 and 3, which are equidistant from each other

Parameter	Sampling point 1	Sampling point 2	Sampling point 3	Average of the analyzed area
Turbidity (*Secchi* disk)	0.70 m	0.70 m	0.75 m	0.72 m
Temperature	28 °C	27 °C	27 °C	27 °C
pH	8	7	7	7

It is important to clarify that, when relating the physical data found (Table 1), some ecosystem conditions are complemented by the physicochemical parameters (Table 2), which suggest that certain environmental characteristics affect the growth and development of biological diversity (Gao et al., 2015). Furthermore, by conducting composite sediment sampling at each point or treatment, the variable of high and low tide conditions could be controlled. Additionally, the ecosystem under study did not exhibit the same depths documented by other researchers (Table 2), providing a broader perspective on the biological behavior of this ecosystem.

Meanwhile, sediments in aquatic environments play a crucial role in managing petroleum-derived hydrocarbon pollution, as they function as a significant sink for organic compounds. In other words, they have the capacity to absorb and retain pollutant compounds, thereby altering the physicochemical parameters of the affected ecosystems (Buskey et al., 2016; Echeverri-Jaramillo et al., 2010).

Insulation

In treatments T1, T2, and T3, color changes were observed in the liquid culture medium compared to the negative controls with sterile water, minimal saline medium, and hydrocarbons. In T1, brown and reddish suspended particles were noted. In T2 and T3, a white and reddish flocculus appeared in the supernatant, indicating color changes that suggest biological activity at the three sampling points. However, to confirm hydrocarbon degradation, quantification methodologies like those performed by Chen et al. (2023b) must be applied. Nevertheless, the bacterial growth observed suggests hydrocarbon transformation.

Table 2. Physical parameters reported in various studies conducted in Turbo Bay, Antioquia

Parameter	Tide	Result	Reference
OD (mg/L)	Not applicable	0.6	Gómez-Cataño et al. (2007)
OD (mg/L)	Low	3.8	Salas-Tovar and Murillo-Hinestroza (2013)
	High	2.4	
Conductivity (µs/cm)	Low	15,749.0	
	High	26,752.0	
Dissolved Solids (mg/L)	Low	8892.0	
	High	16,256.6	
	Not applicable	37.0	Gómez-Cataño et al. (2007)

Additionally, T5 showed a slight color change, indicating the presence of the hydrocarbon ring. The medium's reddish color in the upper part led to the formation of a fuchsia-colored ring, suggesting that the unsterilized seawater may contain a biological component that interacted with the hydrocarbon, as well as biosurfactants described by Bilen-Ozyurek (2023) and Rabelo-Florez & Márquez-Gómez (2020). For T6 with *Pseudomonas aeruginosa*, used as a positive control, a tonal change was observed with the presence of suspended particles, indicating biomass activity potentially related to hydrocarbon ring degradation. This behavior, similar to that seen in treatments T1, T2, and T3, suggests partial hydrocarbon degradation by microorganisms present in the first three treatments (Chen et al., 2023a; Galindo-Soza, 2018).

Conversely, T4 did not show any color change in the medium, nor were suspended particles present. Similarly, T7 and T8 did not exhibit changes in the medium, nor was the hydrocarbon ring deformed; it remained fuchsia in color and did not adhere to the vessel walls. This result confirms the biological activity in the other treatments, where medium alterations were observed, suggesting a possible potential or tolerance by these microorganisms to this contaminant (Chen et al., 2023a; Galindo-Soza, 2018; Echeverri-Jaramillo et al., 2010).

Consequently, 11 morphotypes with macro- or microscopically distinct characteristics were successfully isolated from the three treatments during the selective pressure process (Table 3).

Of the 11 morphotypes, nine were identified as Gram-negative, with bacilli and cocci structures, consistent with the findings reported in the literature (Table 4). Meanwhile, the Gram-negative cocci identified (Table 3) are commonly described as pathogenic, which aligns with the contamination of the ecosystem by organic compounds and domestic and industrial wastewater, leading to a high diversity of pathogenic microorganisms in the environment (Chirre-Flores et al., 2019; Gómez-Mellado et al., 2020; Narváez-Flórez et al., 2008).

Similarly, in morphotypes six and nine, which are described in the literature as pathogens, it can be inferred that the ecosystem's conditions favor the growth of these species, allowing them to adapt and survive in polluted environments. Studies on hydrocarbon biodegradation, such as those by Guzmán and Miluska (2002), suggest that these microorganisms could thrive under favorable conditions, potentially forming a viable microbial consortium. Carrasco-Cabrera (2007) also reported the presence of Gram-negative cocci, identified using the BIOLOG system®. Both cases align with the present study, where Gram-negative bacilli predominated, with a smaller proportion of Gram-negative cocci. However, we recommend conducting monitoring at different times of the year to confirm this observation.

Given that hydrocarbons are a diverse group of toxic compounds with complex structures, it is likely that microbial populations in contaminated areas include a wide variety of bacterial genera. These genera may work together in a consortium to degrade and utilize the pollutant as a substrate (Wang & Tam, 2011).

According to the above, the morphotypes identified, based on their macro- and microscopic characteristics, correspond to the descriptions of cultivable microorganisms isolated from sediments

Table 3. Micro and macroscopic description of isolated morphotypes with their respective coding according to treatment

NO.	Coding	Description Microscopic	Description Macroscopic
1	T1 -3.1 morpho 1	Gram-negative bacilli	Large (extensive in the petri dish) white CFU with a dry texture
2	T1 -3.1 morpho 2	Gram-positive cocci, grouped	Colonies with a defined white border and creamy texture
3	T1 -4.2 morpho 3	Gram-positive cocci	Raised colonies creamy white at the rim and reddish in the center
4	T1 -4.3	Gram-negative bacilli	Growth of different white and creamy convex-shaped colonies is observed
5	T1 -5.1	Gram-negative short bacilli	Creamy white colonies on the edge and reddish in the center
6	T2 -4.1	Gram-negative cocci	White-colored colonies, with rhizoid growth
7	T2 -4.2	Chain bacilli with white spores in the interior Gram-negative	Creamy white colored colonies around and light brown in the center
8	T2 -5.1	Gram-negative short bacilli	Large beige colony with curly growth
9	T2 -5.3	Gram-negative cocci	Growth of isolated creamy white colonies at the edge and reddish in the center
10	T3 -3.2	Gram chain-negative bacilli, colonies appear to be dry	Large transparent colonies with a production of exopolysaccharides
11	T3 -3.2	Gram-negative filamentous bacilli	Medium-sized, white colonies with dry texture and defined edge

in the Caribbean and Pacific regions of Colombia, as reported by Narváez-Flórez et al. (2008). Similarly, using a methodology comparable to that employed in the present study (Abubakar et al., 2024; Varjani, 2017), microbial genera with hydrocarbon biodegrading capacity, such as *Klebsiella* sp., *Flavimonas* sp., *Ralstonia* sp., *Brevibacillus* sp., *Bacillus* sp., *Pseudomonas* sp., *Kluyvera* sp., *Acinetobacter* sp., *Rahnella* sp., and *Stenotrophomonas* sp., have been identified, along with *Rhodococcus* spp. (Braibant-Wayens, 2004; Chen et al., 2023a; Cui et al., 2016).

These bacteria and their activity in the degradation of organic compounds have been previously described in consortia, supporting the recommendation to use mixed cultures in biodegradation processes. The present study, however, was limited to determining the potential or tolerance of microorganisms to hydrocarbons (Gao et al., 2015). In studies related to the degradation of hydrocarbons, Proteobacteria is the dominant taxonomic group found both in phylogenetic analyses and in culturable bacteria, with other taxa such as Firmicutes, Actinomycetota, and Bacillota also being identified. Consequently, various studies have highlighted genera such as *Pseudomonas* sp., *Erythrobacter* sp., *Glaciecola* sp., and *Alteromonas* sp., as well as the class Gammaproteobacteria with Gram-negative staining, as the most common in the isolates found in the present research (Ramírez et al., 2020).

Additionally, the literature review related to this research (Table 4) indicates that the natural physicochemical conditions of the Colombian territory generally favor the proliferation of microorganisms with hydrocarbon biodegrading potential. This suggests that the species reported by various authors are likely also present in the three sites analyzed, given the similar natural conditions and anthropogenic activities along the Caribbean coastal zone. However, it is essential to consider climatic conditions at the time of future sampling, as these may influence changes in population density or the types of potential hydrocarbon-degrading microorganisms present (Gómez et al., 2006; Wang & Tam, 2011).

Regulations

The regulations analyzed and described in this research (Table 5) indicate that Colombia has established legislation to regulate and protect marine-coastal ecosystems, aiming to prevent factors such as excessive dumping that negatively impact biodiversity and to implement mechanisms for conservation. At the district level, and in alignment with national regulations, the municipality of Turbo is actively working to restore its coastal areas. This includes physical actions such as the removal of marine sediment and infrastructure projects in adjacent areas, along with efforts toward landscape beautification and public awareness or education aimed at ecosystem preservation, as outlined in the Development Plan *Turbo Ciudad Puerto* (Turbo Port City). Concurrently, the Corporation for the Sustainable Development of Urabá, in collaboration with the Institute of Marine and Coastal Research (INVEMAR), has established the RedCAM program, a tool for monitoring and tracking the physicochemical parameters of marine waters in the Gulf of Urabá (INVEMAR, 2023a). Data from this program confirm the presence of hydrocarbons in the coastal areas of Urabá, Antioquia (Vivas-Aguas & Navarrete-Ramírez, 2014). However, despite the availability of validated technical data supporting the need for research, no specific policies promoting the bioremediation of these ecosystems were identified at the local level.

Given the results of this research, which led to the isolation of species with biodegradation potential, and in line with the new Colombian policy directions outlined in the *Colombia Potential for Life Development Plan*, this study contributes to the development of new knowledge that will serve as a solid foundation for future interventions focused on the bioremediation of marine-coastal ecosystems affected by hydrocarbon derivatives.

It is important to note that during our investigation, challenges were encountered due to climate variations on the day of sampling. Therefore, it is recommended that future research include monitoring stations to analyze the behavior of these species over time. Nonetheless, the findings reported can be utilized in environmental health strategies to mitigate the impact of potential spills and contribute to the promotion of the protection, recovery, and conservation of marine-coastal ecosystems. This includes accounting for the presence of microbial species or consortia that inhabit the Colombian coasts with this specific physiological potential.

Table 4. Research articles related to the search for microorganisms with hydrocarbon biodegradation potential

Authors	Year	City / Place	Type of methodology applied	Microorganisms reported
Gómez et al.	2006	Caribbean and Colombian Pacific	Isolation, counting, and preservation	*Klebsiella* sp., *Ralstonia* sp., *Brevibacillus* sp., *Bacillus* sp., *Pseudomonas* sp.
Wang and Tam	2011	Yiu Lian's No. 3 Floating in Hong Kong	Isolation in Minimal Salt Medium (MSM) and electrophoresis	Gram-negative bacteria
Dell'Anno et al.	2012	Port of Ancon	Biostimulation	*Chloroflexi*, *Firmicutes*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Verrucomicrobia*
Ganesh-Kumar et al.	2014	Bay of Bengal	Insulation in MSM	*Pseudoalteromonas* sp., *Rugeria* sp., *Exiguobacterium* sp. and *Acinetobacter*
Gao et al.	2015	South Mid-Atlantic Ridge	Biostimulation	Phyla, Proteobacteria, Actinobacteria and Firmicutes
Sukhdhane et al.	2019	Thane Creek, Mumbai	Insulation in MSM	*Bacillus mojavensis*, *Bacillus firmus*, *Bacillus flexus*, *Bacillus vietnamensis* and *Bacillus amyloliquefaciens*
Hamdan et al.	2019	Beirut Coast, Lebanon	Biostimulation	*Proteobacterias*, Genera: *Arthrobacter* sp., *Glaciecola* sp., *Psychrosphaera* sp., *Alteromonas* sp. and Pseudomonas sp.
Ramírez et al.	2020	Gulf of Mexico	Isolation and genetic identification	Phylum Proteobacteria with *Gammaproteobacteria* as the dominant genus other *Firmicutes*

CONCLUSIONS

This study suggests that in the El Waffe dock located in the District of Turbo, Antioquia, there are bacteria that exhibit tolerance to growth in hydrocarbon media composed of a mixture of commercial gasoline and motor oil. The tolerance of these culturable bacteria presents an area of interest for evaluating potential biodegraders of petroleum-derived compounds, with possible applications for bioremediation processes in the marine-coastal environment of the Caribbean and Colombian Pacific contaminated by petroleum.

At the national level, there are environmental guidelines aimed at supporting research projects, including the generation of new knowledge for the preservation of ecosystems or habitats, bioprospecting, and bioeconomics. These guidelines promote the collection of information and the use of this knowledge to develop biological products for the remediation of contaminated environments, as well as measures for mitigating negative environmental impacts and conserving biodiversity.

Conversely, at the district level, there are no projects

Table 5. Regulations or policies related to hydrocarbons in force in Colombia

Name of document	Validity	Authors	Line or Focus of Interest
National Development Plan Colombia, World Power of Life	2022-2026	National Planning Department (2022)	Chapter 4, Catalyst A, paragraph b. Conservation of the natural heritage by strengthening strategies to avoid the alteration and destruction of its protected areas and strategic ecosystems will advance in their restoration
Law 99 of 1993	1993-Present	Congress of Colombia	Establishes the legal framework for environmental management in Colombia and establishes the protection and recovery of coastal ecosystems as a priority for the country
Decree 1076 of 2015	May 26, 2015	Ministry of Environment and Sustainable Development	Article 2.2.3.3.4.14. Contingency Plan for the Management of Hydrocarbon Spills or Harmful Substances
Resolution 1207 of 2014	July 25, 2014	Ministry of Environment and Sustainable Development	Establishes the maximum permissible values for the discharge of treated and wastewater
CONPES 3990 - Consejo Nacional de Política Económica y Social (National Council for Economic and Social Policy) Republic of Colombia	2020-2030	National Planning Department - DNP (2020)	Colombia Sustainable Bioceanic Power 2030. Maritime knowledge, research and culture Line 3
First Update of the National Disaster Risk Management Plan 2022	2015-2030	National Unit for Disaster Risk Management [UNGRD]	Phenomenon: Technological origin. Associated event: Oil spills
Corpouraba Institutional Action Plan	2020-2023	Corpouraba	Strategic Line 2. Biodiversity Conservation and Ecosystem Services
Resolution 0704 of 2020	2020-2030	General Maritime Directorate - DIMAR	Organized by the Caribbean Oceanographic and Hydrographic Research Center and the Pacific Oceanographic and Hydrographic Research Center
Turbo Port City Development Plan	2020-2023	Turbo District Mayor's Office	Pillar 2 - Turbo City Port - Zone 1 Future City
PICIA Four-Year Institutional Plan for Environmental Research	2023-2026	INVEMAR (2023b)	Diagnosis of environmental research and information needs

directly related to the biological remediation of contaminated ecosystems. Current efforts are limited to physical and temporary interventions, such as the removal of marine sediment from the affected coastal area and landscape transformation to facilitate maritime transit. Consequently, there have been minimal changes in the physicochemical parameters studied in the waters of the El Waffe dock in Turbo.

Finally, given the limited existing biological data from sites like the El Waffe dock, this study provides a foundation for future research on the microbiological composition of marine-coastal zones in Urabá Antioquia. It also highlights the potential applicability of bioremediation processes for hydrocarbon-derived compounds and the implementation of microbial consortia techniques for the recovery of these valuable ecosystems.

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AUTHOR CONTRIBUTION

Luisa María Múnera-Porras and John Alexander García-Giraldo provided methodological guidance and advice throughout the research process. Additionally, Múnera-Porras contributed to the writing and proofreading of the manuscript. Aldair Banguera-González and Luis Alberto Jiménez-Medina proposed the research topic and were responsible for its implementation, including experiments, sampling, in situ analysis, and laboratory processing. They also handled the writing and editing of the text. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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