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CHARACTERIZATION OF A *Microbacterium* sp. STRAIN ISOLATED FROM SOILS CONTAMINATED WITH HYDROCARBONS IN THE BURGOS BASIN, MEXICO

Caracterización de una cepa de *Microbacterium* sp. aislada en suelos contaminados con hidrocarburos de la cuenca de Burgos, México

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Key words: *M. petrolearium*, polycyclic aromatic hydrocarbons, bioremediation.

ABSTRACT

The development of novel bioremediation strategies has focused on the isolation and identification of microorganisms that can thrive in polluted environments to evaluate their potential as biotechnological tools in bioremediation techniques. In this work, a bacterium isolated from hydrocarbon-contaminated soils from the Burgos basin was identified and its hydrocarbon degradation potential was evaluated. Identification based on sequencing the 16S rRNA gene identified one of the isolates (R3) as *Microbacterium petrolearium*. This strain was mainly antibiotic-sensitive with elevated carbohydrate assimilation differing from previously reported strains. Moderate surfactant production (I24 = 22.97 %) was observed, which was absent in a cell-free extract. *M. petrolearium* R3 showed increased growth that correlated with pollutant concentration. For light crude oil, at a higher contaminant percentage, the R3 strain showed increased growth; however, in the case of diesel, no growth was detected. The aforementioned data indicate that *M. petrolearium* strain R3, isolated from local sources, has potential use as a tool for hydrocarbon-contaminated soil bioremediation.

Palabras clave: *M. petrolearium*, hidrocarburos aromáticos policíclicos, biorremediación.

RESUMEN

El desarrollo de nuevas estrategias de biorremediación se ha enfocado al aislamiento e identificación de microorganismos que puedan desarrollarse en ambientes contaminados, para evaluar su potencial como herramientas biotecnológicas en técnicas de biorremediación. En el presente trabajo, una bacteria aislada de suelos contaminados con hidrocarburos en la cuenca de Burgos fue identificada y su potencial para degradar hidrocarburos fue evaluado. La identificación basada en la secuenciación del gene del RNAr 16S sobre el aislado R3 indicó a *Microbacterium pretrolearium*. Esta cepa fue sensible a antibióticos con una asimilación de carbohidratos que difiere de cepas reportadas previamente. Se observó una moderada producción de surfactante (I24 = 22.97 %), el cual no se detectó en los extractos libres de células. *M. petrolearium* R3

mostró un crecimiento incrementado en correlación con la concentración del contaminante. Para petróleo crudo ligero, a mayor concentración la cepa R3 mostró mayor crecimiento; sin embargo, en el caso del diésel no se detectó crecimiento. Los datos anteriores indican que *M. petrolearium* R3, aislada a partir de fuentes locales, tiene potencial para su uso en biorremediación de suelos contaminados con hidrocarburos.

INTRODUCTION

In the development of oil-derived products, petrochemical contamination is a constant source of residues that represent a risk for the ecosystem and the exposed population (Reyes-Reyes et al. 2018). Although several techniques have been used to remedy this contamination, bioremediation is widely considered effective and environmentally friendly. This has increased interest in the study of microorganisms that grow in contaminated ecosystems to evaluate their biotechnological potential (Muthukamalam et al. 2017). Biosurfactant release is a metabolic strategy used by microorganisms in the adsorption of hydrocarbons and hydrophobic compounds in general (Johnsen et al. 2005, Chong and Li 2017). It facilitates hydrocarbon degradation (Hmidet et al. 2017, Nievas et al. 2008). Numerous bacteria, such as *Pseudomonas* sp. (Singh and Tiwary 2017), *Microbacterium* sp. (Muthukamalam et al. 2017), *Burkholderia* sp. (Liu et al. 2019), and *Bacillus* sp. (Hmidet et al. 2017) degrade xenobiotics; however, since many are potential human pathogens, they can represent a health risks, making their use complicated.

Contaminated environments represent an opportunity for detecting strains that are ideal for potential bioremediation. A strain of *Microbacterium* was isolated from a sample of water contaminated with oil from Dagan, China (Wang et al. 2014). This strain, based on its phenotypic and genotypic features, was reported as a new species called *Microbacterium petrolearium* LAM0410. It demonstrated an ability to degrade light crude oil in in vitro conditions (Wang et al. 2014). Species of the gender *Microbacterium* can be isolated from diverse environmental sources, among these, the rhizosphere (Wongbunmak et al. 2017) and soil contaminated with polycyclic aromatic hydrocarbons (Sheng et al. 2009).

Hydrocarbon contamination at the Burgos basin, located in northern Tamaulipas, Mexico, has become a relevant problem. As an alternative for the solution and recovery of contaminated environments, diverse techniques that involve microorganisms have been proposed. Therefore, the isolation of microorganisms with the ability to grow in contaminated soil was carried out as a possible alternative for reducing environmental damage. One of the microorganisms isolated was a *Microbacterium* strain that has been previously reported with a potential for hydrocarbon biodegradation (Wang et al. 2014). Since some strains may present physiological differences according to the environment they adapt to, the objective of this work was to characterize and test the biotechnological potential of this strain for hydrocarbon bioremediation.

MATERIALS AND METHODS

Isolation

Sampling was carried out at the Burgos basin in the state of Tamaulipas. The geographic coordinates of the sampling site were 26º 0’ 51” N and 98º 29’ 45” W. The collection method used was a zigzag tracing five different points, collecting 1 kg of soil from each sample point. The soil was then homogenized to obtain a single sample. One gram of sample was mixed by mechanical shaking in a vortex mixer with 10 mL of sterile saline solution (0.85 % NaCl) and left to rest for 10 min.

Afterwards, serial dilutions of 10^{-1} to 10^{-5} of the supernatants were placed in peptone-yeast extract (PY) medium and incubated at 30 ºC for 48 h until growth was observed. Colonies were selected by phenotype to isolate the different types that could grow and were placed individually in PY medium until pure strains were obtained; these were then conserved in glycerol at −80º C (Dasgupta et al. 2013, Reyes-Reyes et al. 2018).

Bacterial identification by RFLP-sequencing

DNA was extracted with the Wizard Genomic DNA purification kit (Promega) according to the manufacturer’s instructions. Amplification of the 16S rRNA gene was performed in triplicate using the universal oligonucleotides, 27f (5’- GAG AGT TTG ATC CTG GCT CAG), and 1495r (5’- CTA CGG CTA CCT TGT TAC GA) (Grifoni et al. 1995). The PCR product was digested with *Alu*I enzyme (buffer

1 X, BSA 1 X, *Alu*I 0.5 U, PCR product 3 µL, for a total volume of 10 µL) (Grifoni et al. 1995). The products were run in 1 % agarose gel, which allowed the observation of restriction patterns. The digestion patterns of the isolates were grouped according to the similarity of each. After the groups were formed, one representative of each was chosen for sequencing. This was performed with an Applied Biosystems model 3130 sequencer using the Sanger method. After obtaining the sequences, multiple alignments were performed in triplicate of the DNAr 16S gene with the Geneious program (v. 4.8.5). The sequences were compared with the reference sequences available in the National Center for Biotechnology Information (NCBI) database using BLAST.

Sequence analysis

To observe the phylogenetic relationship of the *Microbacterium petrolearium* strain R3 with *Microbacterium petrolearium* LAM0410 and nearby species, a phylogenetic tree was constructed using the sequences of the 16S rDNA gene with the neighbor-joining method with 1000 bootstrap replications. The evolutionary distances were calculated with the 3-parameter Tamura method. A phylogenetic analysis was carried out with the MEGA7 program. Sequences of different species of *Microbacterium* were used. These were *Herbiconiux flava* NBRC 16403T (AB583921) and *Leucobacter aerolatus* Sj 10T (FN597581) as external groups. Sequences were obtained from the NCBI database.

Antibiotic resistance

An antibiogram was performed using the agar disk diffusion method (CLSI 2018) with sensi-discs (Bayer and BB Sensi-Disc) for gram-positive bacteria. The antibiotics used were cephalothin, cefotaxime, cefepime, cefuroxime, dicloxacillin, tetracycline and cefoxitin 30 µg, erythromycin 15 µg, ampicillin and gentamicin 10 µg, levofloxacin 5 µg, trimethoprim-sulfamethoxazole 23.75/1.25 µg, and penicillin 10 U.

Isolated colonies were selected from a PY culture plate incubated for 24 h at 30º C. From this, a direct suspension prepared in saline solution was adjusted to 0.5 McFarland and inoculated on the dry surface of the plate ensuring a uniform distribution of the inoculum. Individual discs were placed on the agar surface and incubated at 30º C. After the recommended incubation time, the plates were examined and the inhibition halo diameters around each disc were measured according to Clinical and Laboratory Standards Institute guidelines (CLSI 2018).

Biochemical profile analysis

The strain was assessed using API 20 NE and API 50 CH (bioMérieux, Marcy l'Etoile, France) to determine its biochemical profile and carbohydrate fermentation. This was done according to Wang et al. (2014) in the *Microbacterium petrolearium* strain LAM0410. The analysis was carried out according to the manufacturer's instructions reading the results after 24 h of incubation.

Evaluation of biosurfactant production

Hemolytic activity

This evaluation was performed according to Carrillo et al. (1996). The strains were seeded on plates with a blood agar base (MCD LAB) (40 g/L) with 50 mL/L of sheep blood and incubated for 48 h at 30º C (SHEL-LAB model 1545 incubator). A strain of *Staphylococcus aureus* was used as a positive control and the evaluation was performed in triplicate.

Synthesis of a rhamnolipid biosurfactant

The rhamnolipid biosurfactant was detected using the technique reported by Siegmund and Wagner (1991). To perform the evaluation, a drop of culture grown for 22 h (stationary phase) was deposited on a Petri dish with S-W medium (0.2 g cetyl trimethyl ammonium bromide [CTAB], 0.005 g methylene blue, 20 g glucose, 0.7 g KH_2PO_4, 0.9 g Na_2HPO_4, 2 g $NaNO_3$, 0.4 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot H_2O$, 15 g agar and 2 mL of trace elements [H_3BO_3 0.26 g/L, Na_2MoO_4 0.6 g/L, $CuSO_4 \cdot 5H_2O$ 0.5 g/L, $MnSO_4$ 0.5 g/L, $ZnSO_4 \cdot 7H_2O$ 0.7 g/L] per liter of deionized water). The plates were incubated for 48 h at 30º C (SHEL-LAB model 1545 incubator) to observe the formation of a complex between CTAB and methylene blue, revealed by the presence of a dark blue halo around the drop of culture. In this evaluation, *Sphingobium yanoikuyae* S72 was used as a positive control; assays were performed in triplicate.

Percentage of the emulsification index (E24)

This was measured using the method described by Illori et al. (2005). To evaluate the emulsification index, *Sphingobium yanoikuyae* S72 was used as the positive control strain and 10 % Tween 80 as the positive control for emulsion; the evaluation was performed in triplicate. The emulsifying activity was measured by adding 2 mL of crude oil to 2 mL of a cell-free extract and mixing them in a vortex at high velocity for 2 min. This same procedure was performed using a medium with bacterial cells and a cell-free extract. The emulsion was measured at two times (1 h and 24 h). In both cases, the emulsification

index (E24) was obtained by measuring the height of the emulsification divided by the total height multiplied by 100.

Evaluation of light crude oil and diesel as a carbon source

A colony of the strain was inoculated into tubes with 4 mL of PY medium for *Microbacterium petrolearium* R3. The tubes were incubated with shaking (SHEL-LAB model 1545 incubator) for 24 h to obtain a bacterial biomass with an optical density of 0.4. Finally, 1 mL of the culture was transferred to Eppendorf tubes, centrifuged, and washed twice with 1 mL sterile saline. From this stock, 1.5×10^5 CFU was inoculated into each of the flasks that contained 30 mL of Bushnell-Haas minimal salts medium ($MgSO_4$ 0.2 g/L, $CaCl_2$ 0.02 g/L, KH_2PO_4 1 g/L, K_2HPO_4 1 g/L, NH_4NO_3 1 g/L, $FeCl_3 \cdot 05$ g/L) using two concentrations (1 and 5 %) of light crude oil and diesel as the sole carbon source. The flasks were incubated with shaking (SHEL-LAB model 1545 incubator) at 30º C for 30 days; during this period, OD was measured daily at 600 nm. For this, Bushnell-Haas medium with the contaminant and without the inoculum was used as the negative control to consider possible abiotic loss (Dasgupta et al. 2013).

RESULTS

Isolation

After 48 h of incubation at 30˚ C, colonies were selected according to morphology, color, texture, and size. These were spread onto Petri dishes with PY culture medium until pure isolates were obtained. A total of 17 pure isolates were obtained; their characteristics are listed in **table I**.

TABLE I. COLONY MORPHOLOGY OF THE PURE ISOLATES OBTAINED.

Strain	Color	Colony morphology	Strain	Color	Morphology
R01	Orange	Smooth	R11	Beige	Rough
R02	Yellow	Rough	R12	Yellow	Smooth
R03	Yellow	Rough	R13	Beige	Rough
R04	Beige	Smooth	R14	Orange	Smooth
R05	Beige	Smooth	R15	Beige	Rough
R06	Yellow	Smooth	R16	Beige	Rough
R07	Orange	Smooth	R17	Beige	Smooth
R08	Orange	Smooth	R18	Yellow	Smooth
R09	Beige	Rough	R19	Beige	Smooth
R10	Beige	Smooth			

Bacteria identification

Ten isolates were sequenced according to their different enzyme digestion patterns. Once acquired, alignment results performed with BLAST allowed the identification of bacteria, as shown in table II. The R3 strain was identified as *Microbacterium petrolearium* (**Table II**).

TABLE II. RESULTS OF THE MOLECULAR IDENTIFICATION OF THE ISOLATES OBTAINED.

Isolate	Percentage of similarity	Identification
RO1	93	*Rhodococcus ruber*
RO2	97	*Micrococcus luteus*
RO3	95	*Microbacterium petrolearium*
RO4	94	*Microbacterium marinilacus*
RO5	93	*Staphylococcus haemolycticus*
RO6	98	*Bacillus subtilis*
RO7	98	*Bacillus pumilus*
R08	94	*Micrococus luteus*
R09	96	*Bacillius methylotrophicus*
R10	98	*Arthrobacter* sp.

Of the isolates identified by sequencing, only the R3 strain (*Microbacterium petrolearium*) was selected for the next part of the study. This was because this new species of the genus *Microbacterium***,** reported by Wang et al. (2014), was isolated from soil contaminated with hydrocarbons. Therefore, we considered that its characterization could provide information regarding its potential use in studies related to xenobiotic degradation.

Sequence analysis

The resulting phylogenetic tree showed a similarity of 100 % with the *Microbacterium petrolearium* strain R3 and the *M. petrolearium* strain LAM0410; in other words, both strains belong to the same species. This association is followed by a similarity of 99 % with *Microbacterium sediminis* YLB-01 as a close species, and it establishes itself as a group external to *Herbiconiux flava* and *Leucobacter aerolatus* (**Fig. 1**).

Antibiotic resistance

Microbacterium petrolearium R3 was resistant to dicloxacillin 30 µg, with intermediate resistance to cefuroxime 30 µg, and sensitive to ampicillin 10 µg, cephalothin 30 µg, cefotaxime 30 µg, cefepime 30 µg, cefuroxime 30 µg, erythromycin 15 µg,

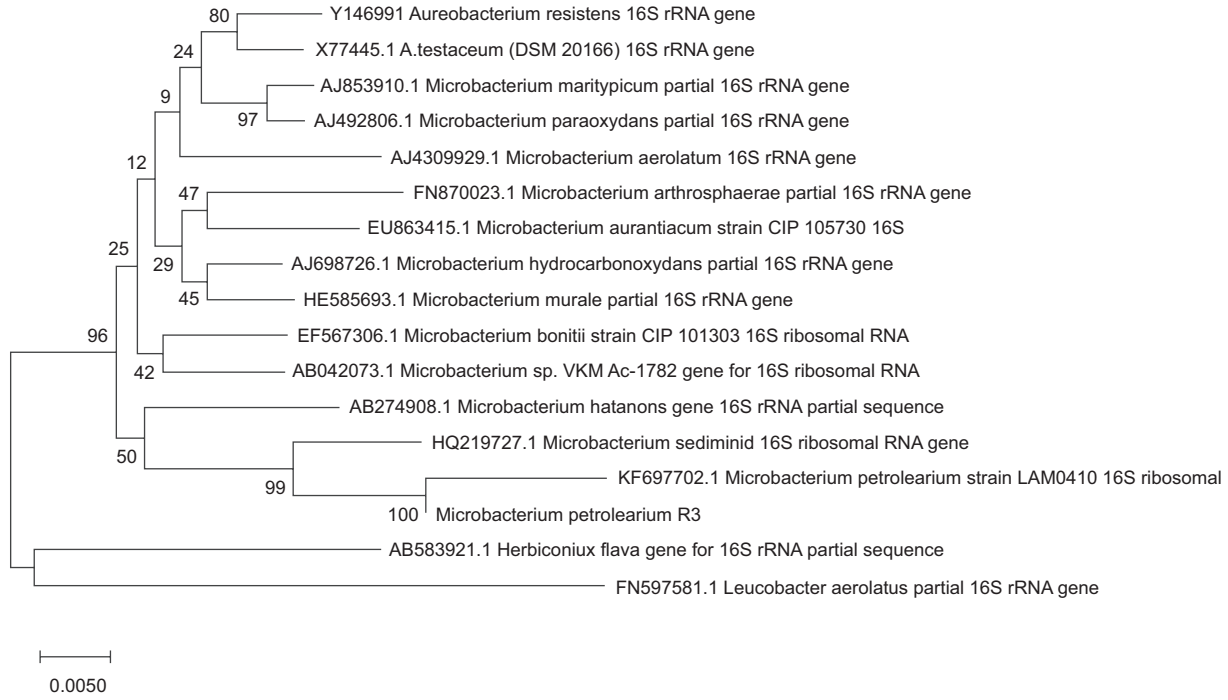

Fig. 1. Phylogenetic tree of *Microbacterium petrolearium* R3 made using the neighbor-joining method with a bootstrap of 1000 replications. The evolutionary distances were calculated with the 3-parameter Tamura method.

gentamicin 10 µg, levofloxacin 5 µg, penicillin 10 U, tetracycline 30 µg, trimethoprim-sulfamethoxazole 23.75/1.25 µg, and cefoxitin 30 µg.

Biochemical analysis (API)

The results of API 20 NE and API 50 CH were analyzed after 24 h of incubation. The API 20 NE evaluation showed that both strains (LAM0410 and R3) were negative for the reduction of nitrates, tryptophan, urea, arginine, and gelatin. Differences were observed in the assimilation of carbon sources, such as esculin and 4-nitrophenyl-βD-galactopyranoside, with these being positive for *M. petrolearium* R3 and negative for *M. petrolearium* LAM0410, while LAM0410 was positive for D-maltose, and L-arabinose was negative for the R3 strain.

In the case of API 50 CH, the *Microbacterium petrolearium* strain R3 was able to use D-fructose, inositol, amygdalin, esculin, D-cellobiose, D-sucrose, and 4-nitrophenyl-βD-galactopyranoside as carbon sources. Differences in assimilation were found when comparing the strain LAM0410 since this strain is capable of assimilating D-sorbitol, D-trehalose, L-arabinose, and D-maltose, which were negative for the R3 strain.

Characterization of biosurfactant production

Hemolytic activity and synthesis of a rhamnolipid biosurfactant

In the evaluation of hemolytic activity and a rhamnolipid biosurfactant, the strains *Staphylococcus aureus* and *Microbacterium petrolearium* R3 grew in a blood-based medium; however, *Microbacterium petrolearium* R3 did not present hemolysis. In the analysis of rhamnolipid biosurfactant synthesis, the two strains showed growth; however, only *Microbacterium petrolearium* R3 was positive.

Percentage of the emulsification index (E24)

The percentage of the emulsification index after 1 h was 38.73 % for *Microbacterium petrolearium* R3 and 26.1 % for *Sphingobium yanoikuyae* S72 in the cell-free extract, and 36 % for *Microbacterium petrolearium* R3 and 19.8 % for *Sphingobium yanoikuyae* S72 in the cell culture. After 24 h of incubation, none of the strains presented emulsification in the cell-free extract; however, in the cell culture, the only strain that presented emulsification in 22.97 % was *Microbacterium petrolearium* R3 (**Table III**).

TABLE III. PERCENTAGE OF THE EMULSIFICATION INDEX (E24).

Strain	Percentage of the emulsification index (E24)			
	Cell-free extract		Cell culture	
	1 hour	24 hours	1 hour	24 hours
M. petrolearium	38.73	0	36	22.97
S. yanoikuyae S72	26.1	0	19.8	0
Tween 20	100	100	100	100

Evaluation of light crude oil and diesel as a carbon source

The assays of light crude oil and diesel as a carbon source for *Microbacterium petrolearium* strain R3 produced a growth curve of 30 days. In this, the *Microbacterium petrolearium* strain R3 was evaluated at concentrations of 1 and 5 %, respectively, for both contaminants, light crude oil (**Fig. 2a**) and diesel (**Fig. 2b**) for 30 days.

As seen in both graphs, *Microbacterium petrolearium* R3 shows minimum growth in the absence of either of the two contaminants. In the case of light crude oil, a slight increase in growth is seen at 1 % after 15 days of incubation; however, when the 5 % concentration is used, the growth increase is greater. When evaluating diesel using the concentrations of 1 and 5 %, growth is not observed in comparison to the growth seen with light crude oil. In contrast, growth inhibition is seen with the concentration used; that is, at a higher percentage of the contaminant, there is a greater growth inhibition of *M. petrolearium* R3.

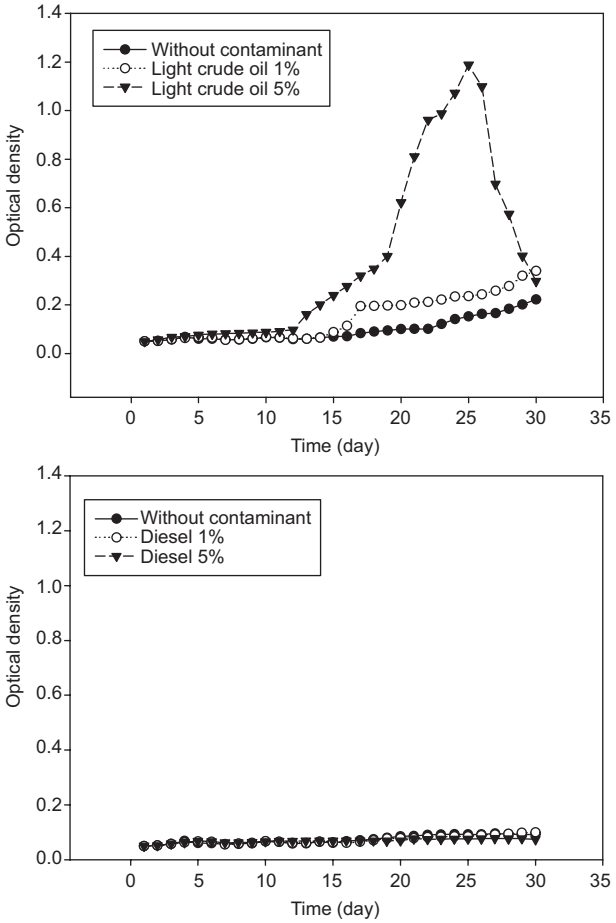

Fig. 2. Growth kinetics of *Microbacterium petrolearium* R3 at concentrations of 1 and 5 % of light crude oil and diesel during a period of 30 days.

DISCUSSION

The role of microbe activity in the biodegradation of hydrocarbons has been known for more than a century. The *Mycobacterium* strain isolated in this study is already adapted to hydrocarbon contaminated soils in the Burgos basin of Tamaulipas.

The analysis of the ribosomal gene 16S showed that the strain R3 belongs to the species *Microbacterium petrolearium* with an identity of 98 % and a coverage of 99 %. This gene sequencing analysis indicated that the *Microbacterium pretolearium* reported by Wang et al. (2014) and our *Microbacterium petrolearium* strain isolated in this work belong to the genus *Microbacterium* and are closely related to *Microbacterium sediminis* MCCC 1A06153T, a thermotolerant, halotolerant and alkali-tolerant strain isolated from deep-sea sediments (Yu et al. 2013). The species of the genus *Microbacterium* have been isolated from a large variety of ecosystems including sites contaminated with xenobiotics and evaluated according to these properties (Camacho-Chab et al. 2013). It is important to mention that it is a recently reported resistant strain; therefore, the fact that it was isolated from soil contaminated with hydrocarbon xenobiotics in this region is of great relevance due to its possible potential to degrade hydrocarbons and its adaption to these conditions, which arise as an alternative for reducing environmental damage.

Microbacterium petrolearium R3 presented resistance to dicloxacillin and intermediate resistance to cefuroxime. It has been reported that bacterial populations can survive in unfavorable environments in the presence of inhibitory substances such as antibiotics and hydrocarbons because of their ability to

produce enzymes that inactivate or modify them and because they interrupt the function of the xenobiotic (Narváez-Flórez et al. 2008). Hydrocarbons are lipophilic compounds that in elevated concentrations inhibit microbe development, produce intoxication, induce a stress response in bacteria, and cell changes at the membrane, enzyme, and protein levels (Lee et al. 2018). The biochemical process of degradation of hydrocarbons by microorganisms involves the action of enzymes, including oxygenases, dehydrogenases, and hydroxylases that fragment aliphatic and aromatic hydrocarbons to transform and mineralize them (Muthukamalam et al. 2017). These organisms are capable of accumulating, transforming, or degrading contaminants to concentrations that do not produce harmful effects on health or the environment (Miranda-Martínez et al. 2007).

The results of the sources of carbon assimilation obtained from the R3 strain compared with those obtained by Wang et al. (2014) with the LAM0410 strain revealed differences because the latter is capable of assimilating D-sorbitol, D-trehalose, L-arabinose, and D-maltose, which were negative for the R3 strain. Wongbunmak et al. (2017) reported that *Microbacterium esteraromaticum* strain SBS1-7 is capable of assimilating L-arabinose. These variations occur because each strain is capable of assimilating carbon sources. The importance of evaluating their assimilation is directly related to the contribution of the nutrients necessary for the growth of the bacteria and that the carbon source used can influence the metabolites produced (Mouafo et al. 2018).

The release of biosurfactants is one of the strategies used by microorganisms to influence the absorption of hydrocarbons and hydrophobic compounds in general (Chong and Li 2017). These are characterized by their low toxicity and are biodegradable in comparison with those of synthetic origin making them an alternative for hydrocarbon remediation (Chong and Li 2017). Thus, they were indirectly characterized.

Initially, the evaluation was carried out measuring hemolytic activity, obtaining a negative result for *M. petrolearium* R3. The determination of hemolytic activity using blood agar is useful for the preliminary selection of bacteria that are potential producers of biosurfactants. Sarti and Miyazaki (2013) reported that *Bacillus subtilis* ATCC6633 released molecules with hemolytic capacity when using lamb blood-enriched agar-based medium, suggesting the production of substances with biosurfactant properties associated with hemolytic activity. However, other reports establish that the determination of hemolytic activity is not considered a definitive method. When evaluating a strain of *Pseudomonas* sp., Pacwa-Płociniczak et al. (2011) reported that the positive result of hemolytic activity of this strain was insufficient to confirm the production of biosurfactants because these also possess biotensioactives and β-hemolysins; therefore, the application of additional tests is required since not all biosurfactants have hemolytic activity. Also, compounds that are not biosurfactants can produce hemolysis.

Microbacterium petrolearium R3 had a positive result in the Siegmund and Wagner (SW) agar test. This test is commonly used for the detection of rhamnolipid biosurfactants; in some studies, it has been used as a semiquantitative test that relates the intensity of the halo with the amount of anionic tensoactive that interacts with CTAB and methylene blue (Becerra and Horna 2016). The SW technique arose as an alternative to the use of blood agar for the detection of biosurfactants since it has several advantages such as the non-catabolic repression of biosurfactant production, the non-occurrence of false positives due to the presence of hemolysins, and the greater stability of the agar (Tabuchi et al. 2015). The qualitative detection of rhamnolipid biosurfactant production in *M. petrolearium* R3 suggests that it possesses mechanisms that could facilitate the bioavailability of hydrocarbons.

In the evaluation of the emulsification index, two treatments were used, a cell-free extract and a cell medium. Twenty-four hours after emulsion, the cell medium presented an emulsification index of 22.97 %, showing greater stability in the emulsion, while the cell-free extract did not conserve emulsion. The results varied depending on the microorganism studied and the carbon source. Illori et al. (2005) evaluated a strain of *Aeromonas* spp. isolated from tropical waters. The biosurfactants produced by the organisms emulsified a range of hydrocarbons obtaining an E24 of 65 % when using diesel as a carbon source and of 22 % when using hexane. In a study by Obayori et al. (2009), when evaluating the potential production of biosurfactants in *Pseudomonas* sp. P1, the E24 was 80.33 ± 1.20 using oil as a carbon source after eight days of incubation. The importance of the carbon sources used in this type of study makes it essential to evaluate the most adequate in assays for each microorganism.

There are different quantitative or qualitative methods available that vary in their precision and the objectives of production tests of potential

biosurfactant-producing microorganisms. Among these, hemolytic activity assays and the CTAB agar method are considered primary assessments for biosurfactant-producing strains; however, more than one evaluation method should be included in the primary selection to identify possible biosurfactant-producing strains (Elazzazy et al. 2015).

Camacho-Chab et al. (2013) evaluated a non-toxic bioemulsifier synthesized by a strain of *Microbacterium* sp. MC3B-10. The bioemulsifier presented an E24 of 76 % for crude oil, showing that some members of the genus *Microbacterium* produce biosurfactants as reported in this study.

The results in the evaluation of the tolerance of *Microbacterium petrolearium* R3 suggest that it could be assimilating light crude oil as its only source of carbon and energy. Muthukamalam et al. (2017) reported a study in which they isolated and characterized a strain identified as *Microbacterium hydrocarbonoxydans* BM. This strain was evaluated in in vitro assays using light crude oil as a carbon source and energy at concentrations of 1, 2, and 3 %. The results showed that this strain presented a greater growth in relation to an increase in the volume of hydrocarbon used, similar to the results obtained in the growth kinetics of the strain *Microbacterium petrolearium* R3 in the presence of crude oil. However, in this work, studies to evaluate the percentage of degradation were not performed; therefore, it is necessary to perform assays that involve the evaluation of percentages of degradation of contaminants by this species. In the case of the results obtained when diesel is added to the culture medium, these suggest that the use of *Microbacterium petrolearium* could be limited or null.

CONCLUSIONS

In this work, the characterization of a bacterium identified as *Microbacterium petrolearium* R3 by ribosomal gene 16S sequencing is presented. This bacterium was sensitive to the tested antibiotics and differed in its ability to degrade hydrocarbons. We observed moderate production of a surfactant (I24 = 22.97 %) that was not detected in the cell-free extract. It was also found that *M. petrolearium* R3 presented a notable increase in its growth in relation to the concentration of the added contaminant. In the case of light crude oil, a greater percentage of added R3 strain showed greater growth; however, for diesel, no growth was detected.

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