AgroLife Scientific Journal - Volume 11, Number 2, 2022
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

# ALIPHATIC HYDROCARBONS BIODEGRADATION BY A *Pseudomonas* STRAIN

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***Abstract***

*The present study aimed to evaluate the potential of a Pseudomonas aeruginosa strain to tolerate and degrade aliphatic petroleum hydrocarbons. P. aeruginosa $IBB_{Ct8}$ was isolated from a seawater sample using crude oil as the sole carbon source in a minimal medium. The bacterium was identified as P. aeruginosa based on its morphological, biochemical, and molecular characteristics. P. aeruginosa $IBB_{Ct8}$, which possesses alkB (870 bp) and rhlAB (216 bp) genes in its genome, showed a higher tolerance and degradation efficiency of the n-hexadecane, n-decane, cyclohexane, or paraffin oil, compared with the tolerance to n-hexane and its degradation. P. aeruginosa $IBB_{Ct8}$ cells produced rhamnolipid biosurfactants which showed a very good emulsification activity against the tested aliphatic hydrocarbons. Due to its characteristics, the isolated bacterium could be used in the bioremediation of petroleum-polluted environments.*

***Key words****: Pseudomonas; aliphatic hydrocarbons, biodegradation.*

## INTRODUCTION

Nowadays, the extraction, transportation, and processing of petroleum (also known as crude oil) still determine serious accidental and chronic pollution of numerous environments (Stancu, 2018a).

Petroleum is a complex mixture of hydrocarbons, such as aliphatic, aromatics, asphaltenes, resins, and most of these compounds are recalcitrant and are classified as high-priority pollutants (Abbasian et al., 2015). Thus, the cleaning up of petroleum-polluted environments is a real-world problem (Stancu, 2018a). It is well known that some of the microorganisms, especially bacteria from the *Pseudomonas* genus that naturally exist in polluted environments, can tolerate and/or degrade toxic petroleum hydrocarbons (Stancu, 2018b; 2019).

Petroleum hydrocarbons are usually toxic for most bacteria since they are partitioned by passive and/or active transport processes in the cytoplasmic membrane.

As a result of hydrocarbon partitioning in the lipid bilayer, modifications in the structure and function of membranes appear (Sikkema et al., 1995). Several adaptive mechanisms, such as modifications of the membrane or changes in the overall energy status, modifications of cell morphology and cell surface properties, active excretion, and induction of anabolic pathways were described for bacteria able to survive in the presence of toxic petroleum hydrocarbons (Sikkema et al., 1995; Eberlein et al., 2018). Bacteria can degrade aliphatic (saturated, unsaturated) hydrocarbons via both aerobic and anaerobic pathways. Branched hydrocarbons and cyclic hydrocarbons are also degraded by several bacteria. The aerobic bacteria use different oxygenase (e.g., monooxygenase, dioxygenase) to insert one or two atoms of oxygen into hydrocarbon molecules, while the anaerobic bacteria, use organic or inorganic molecules (e.g., sulfate, nitrate, carbonate) for hydrocarbon oxidation (Abbasian et al., 2015).

The high interest given worldwide to the research on the study of bacteria able to tolerate and degrade toxic hydrocarbons is because of their high biotechnological potential. The use of bacteria to degrade toxic hydrocarbons into non-toxic end products (e.g., carbon dioxide, water, inorganic compounds) is an environmentally friendly, effective, economic, and versatile alternative to physicochemical treatment of petroleum-polluted environments (Stancu, 2018a). The present study **aimed** to evaluate the potential of a *Pseudomonas aeruginosa* strain to tolerate and degrade several aliphatic hydrocarbons, such as

cyclohexane, *n*-hexane, *n*-decane, *n*-hexadecane, and paraffin oil.

## MATERIALS AND METHODS

### Isolation and characterization of the bacterium

The bacterium used in this study was isolated by enrichment culture method, from a seawater sample collected from Constanta harbor (Constanta County, Romania), using 5% (v/v) crude oil as the sole carbon source in the minimal medium (Stancu and Grifoll, 2011). Here as elsewhere in this work, the assays were done in duplicate. The isolated bacterium was stored frozen at -80°C in 25% (v/v) glycerol.

The bacterium was first characterized by standard morphological and biochemical tests (e.g., Gram reaction, shape, motility, growth onto King A, King B, and TTC agar, respiratory type, nitrate reductase, indole, urease, gelatin, and esculin hydrolysis, etc., Holt et al., 1994). For the molecular characterization, the genomic DNA was extracted from overnight cultures using the Pure Link genomic kit (Invitrogen, Carlsbad, CA, USA). The random amplification of polymorphic DNA (RAPD) was performed using genomic DNA (0.5 µg), a single short primer (AP5, Pini et al., 2007), and GoTaq G2 Flexi DNA polymerase (Promega, Madison, WI, USA) according to the manufacturer's instructions. The PCR program consisted of an initial denaturation step (94°C, 10 min), followed by 45 cycles of amplification (94°C, 1 min, 36°C, 60 sec, 72°C, 2 min), and a final extension step (72°C, 10 min). The reaction products were visualized on 2.0% (w/v) agarose gel (Sambrook and Russel, 2001) after staining with SYBR Safe (Invitrogen).

The PCR amplification of alkane hydroxylase (i.e., *alkB*, *alkM*, *alkB1*, *alkB, alkM1*) genes and rhamnosyltransferase 1 (i.e., *rhlAB*) genes were performed using genomic DNA (0.5 µg), specific primers (ALK1-f/ALK1-r, ALK2-f/ALK2-r, ALK3-f/ALK3-r, Kohno et al., 2002; alkB-f/alkB-r, Whyte et al., 1996; alkM-f/alkM-r, Márquez-Rocha et al., 2005; rhlA-f/rhlB-r, Medina et al., 2003), and GoTaq G2 Flexi DNA polymerase (Promega) according to the manufacturer's instructions. The PCR program consisted of an initial denaturation step (94°C, 10 min), followed by 35 cycles of amplification (94°C, 1 min, 50°C-62°C, 30 sec, 72°C, 2 min), and a final extension step (72°C, 10 min). The reaction products were visualized on 1.5% (w/v) agarose gel (Sambrook and Russel, 2001) after staining with SYBR Safe (Invitrogen).

### Tolerance and degradation of aliphatic hydrocarbons

The tolerance of the bacterium to aliphatic hydrocarbons, such as cyclohexane, *n*-hexane, *n*-decane, *n*-hexadecane, and paraffin oil, was determined by overlay plates assay (Satpute et al., 2008). Hydrocarbons used in this study were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Saint-Quentin-Fallavier, France). Overnight cultures, grown in LB medium at 30°C, were spotted (25 µl, $OD_{660}$ 0.50, 0.25) onto LB agar (Sambrook and Russel, 2001) plates, air-dried, and then overlaid with aliphatic hydrocarbons. Control experiments were set up similarly, but without hydrocarbons. After 4 hours, the hydrocarbons were removed from the top of the Petri plates, and they were sealed and incubated 24-48 hours at 30°C. Hydrocarbon's tolerance was expressed as a function of the bacterial growth, as compared with the control. The Petri plates were visualized under visible light (500 nm) and UV light (366 nm).

The tolerance of the bacterium to the same aliphatic hydrocarbons and their biodegradation potential was further determined by a liquid overlay assay (Stancu, 2020). Overnight cultures grown in LB medium at 30°C, were inoculated ($OD_{660}$ 0.10) in liquid LB (Sambrook and Russel, 2001) and minimal medium (Stancu and Grifoll, 2011), containing 5% (v/v) aliphatic hydrocarbons. Control experiments were set up similarly, but without hydrocarbons. Flasks were sealed and incubated 24-144 hours at 30°C on a rotary shaker (200 rpm). The tolerance of the bacterium to aliphatic hydrocarbons was monitored by determining, at different time intervals, the optical density at 660 nm ($OD_{660}$) using a SPECORD 200 UV-visible spectrophotometer (Analytik Jena, Jena, Germany) and viability of the cell by spot assay (Stancu, 2020). The hydrocarbons biodegradation by this bacterium was evaluated in the minimal medium through gravimetric

analyses of the residual hydrocarbons extracted with chloroform (Gulati and Mehta, 2017).

**Biosurfactants production**

The bacterium was inoculated into the liquid LB medium (Sambrook and Russel, 2001). Flasks were incubated 24-144 hours at 30°C on a rotary shaker (200 rpm). Biosurfactants production by the bacterium was determined through the CTAB agar plate assay (Siegmund and Wagner, 1991), drop-collapse assay (Bodour and Miller-Maier, 1998), emulsification index ($E_{24}$) against aliphatic hydrocarbons (Abdel-Mawgoud et al., 2009), and by HPTLC analyses of the crude surfactants extracted with chloroform-methanol (Gesheva et al., 2010) from the cell-free culture broths. High-performance thin-layer chromatography (TLC) analyses of extracts were completed using a CAMAG thin-layer chromatography (TLC) system (Muttenz, Switzerland) as described by Stancu (2018b). Biosurfactant fractions were separated on TLC precoated silica gel 60 plates (Merck) using chloroform-methanol-water mixture (65:25:4 v/v/v) as mobile phase. After derivatization with iodine vapors to detect lipids or 0.2% orcinol in 53% sulfuric acid to detect sugars in the biosurfactant molecules, TLC plates were visualized and scanned under ultraviolet (UV) light (366 nm) and visible light (500 nm).

## RESULTS AND DISCUSSIONS

**Isolation and characterization of the bacterium**

The strain $IBB_{Ct8}$ isolated through the enrichment culture method by a seawater sample was identified as *Pseudomonas aeruginosa* based on their morphological and biochemical characteristics. The strain $IBB_{Ct8}$ was Gram-negative, motile, rod-shaped cells, and facultatively anaerobic. It was positive for catalase, oxidase, nitrate reductase, L-arginine dihydrolase, urease, gelatin hydrolysis, D-glucose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate assimilation and negative for indole, D-glucose fermentation, esculin hydrolysis, β-galactosidase, L-arabinose, D-mannose, N-acetyl-glucosamine, D-maltose, phenylacetic acid assimilation. The identification of the strain $IBB_{Ct8}$ as *P. aeruginosa* was confirmed by their growth onto three specific culture media, such as King A, King B, and TTC agar (Figure 1).

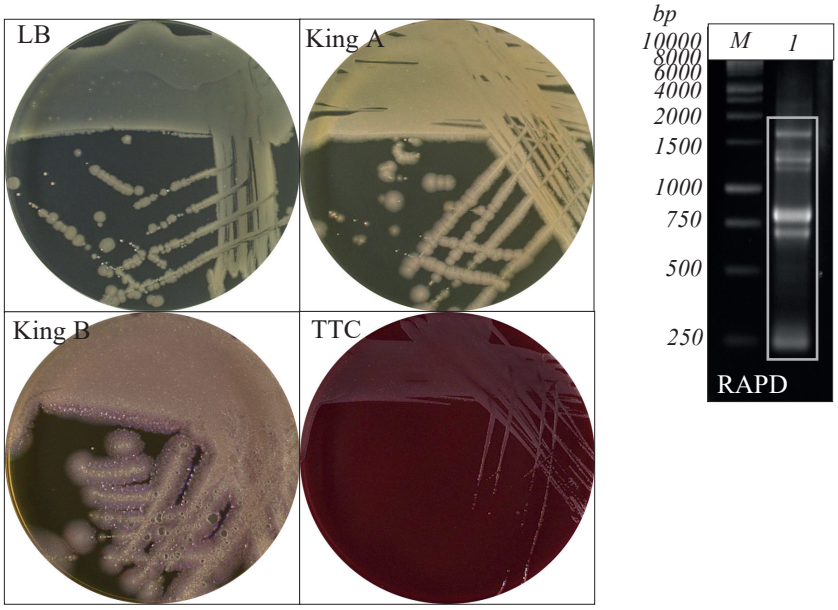
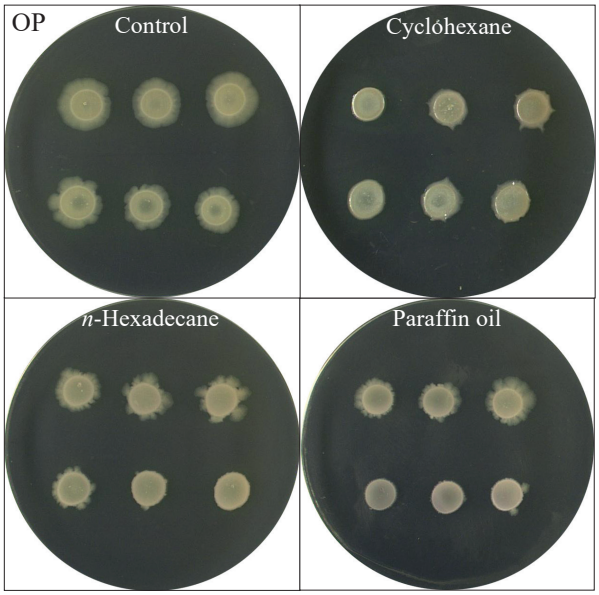



Figure 1. Isolation and characterization of *P. aeruginosa* $IBB_{Ct8}$
Macroscopic view, the bacterium was grown on LB, King A, King B, TTC agar. RAPD using genomic DNA and primer AP5 (*1*), 1 kb DNA ladder, Promega (*M*)

Furthermore, the strain $IBB_{Ct8}$ showed a comparable RAPD fingerprint profile (size range between 250-1800 bp) to that of other strains of *P. aeruginosa* from our lab collection (data not shown). The isolation, from a seawater sample, of a strain of *P. aeruginosa,* able to grow on minimal medium in the presence of crude oil as the sole carbon source, is in agreement with previously obtained data (Muriel-Millán et al., 2019) according to which marine bacteria are well adapted to alkanes degradation that commonly enters in the marine environments from various natural and anthropogenic sources. *P. aeruginosa* isolated from petroleum-polluted environments have greater alkane-degradation capacities than clinical strains, suggesting an evolutionary adaptation of them to hostile conditions from these environments (Muriel-Millán et al., 2019).

**Tolerance and degradation of aliphatic hydrocarbons**

The tolerance of *P. aeruginosa* $IBB_{Ct8}$ to aliphatic hydrocarbons (i.e., cyclohexane, *n*-hexane, *n*-decane, *n*-hexadecane, paraffin oil) was further determined by overlay plates assay. Higher growth was observed when the *P. aeruginosa* $IBB_{Ct8}$ was spotted on LB agar (control) or LB agar overlaid with *n*-hexadecane, *n*-decane, compared with those observed when the bacterium was spotted on LB agar overlaid with cyclohexane, or paraffin oil. In this assay, the *P. aeruginosa* $IBB_{Ct8}$ showed good tolerance to *n*-hexadecane (log $P_{OW}$ = 9.15), *n*-decane (log $P_{OW}$ 5.98), cyclohexane (log $P_{OW}$ 3.35), or paraffin oil, and a lower tolerance to *n*-hexane (log $P_{OW}$ 3.86) (Figure 2). The tolerance of bacteria to toxic hydrocarbon is correlated with their log $P_{OW}$ (logarithm of the hydrocarbons partition coefficient between octanol and water) values (Sikkema et al., 1995). *P. aeruginosa* $IBB_{Ct8}$ cells spotted on LB agar (control) or LB agar overlaid with aliphatic hydrocarbons produced the pyoverdin pigment (fluorescent yellow-green pigment), visible under UV light. A direct correlation between bacterial cell growth and pyoverdin pigment production was observed (Figure 2). Although hydrocarbons with log $P_{OW}$ values between 1 and 5 are usually highly toxic for bacteria, each strain has its intrinsic tolerance level which is environmentally influenced and genetically determined (Torres et al., 2011). The higher tolerance of bacteria to toxic petroleum hydrocarbons is sometimes linked to the outer membrane resistance to hydrophobic compounds, due to the presence of hydrophilic lipopolysaccharides (Sikkema et al., 1995).



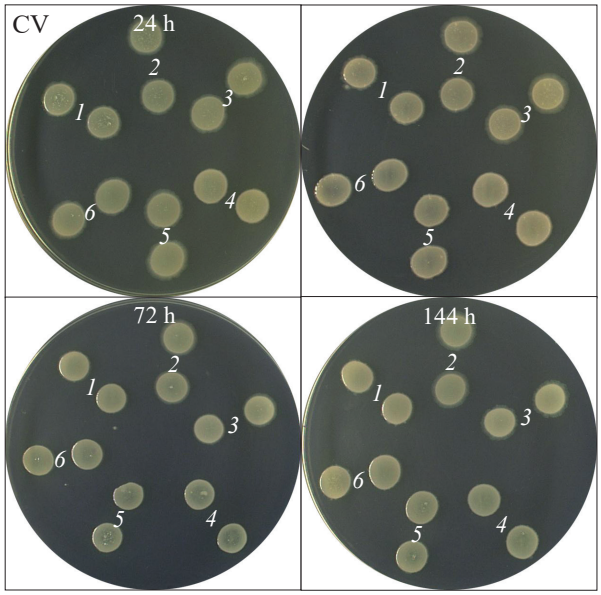

| Hydrocarbon's tolerance | BG (PG) | PvP |
|---|---|---|
| Control | ++ | ++ |
| Cyclohexane | + | + |
| *n*-Hexane | – | – |
| *n*-Decane | ++ | ++ |
| *n*-Hexadecane | ++ | ++ |
| Paraffin oil | + | + |

Figure 2. Tolerance of *P. aeruginosa* $IBB_{Ct8}$ to hydrocarbons

Overlay plates (OP) assay, cultures were spotted onto LB agar and overlaid with hydrocarbons. Bacterial growth (BG), profuse growth (PG) on the overlay plates, pyoverdin pigment (PvP) production on the overlay plates, positive reaction (++, +), negative reaction (–)

The tolerance of *P. aeruginosa* $IBB_{Ct8}$ to 5% aliphatic hydrocarbons (i.e., cyclohexane, *n*-hexane, *n*-decane, *n*-hexadecane, paraffin oil) and their biodegradation potential was further studied. After 24-144 hours of incubation, the growth of *P. aeruginosa* $IBB_{Ct8}$ on liquid LB was higher for the control cells ($OD_{660}$ 0.93-2.93), compared with that acquired for cells grown on the same medium but in the presence of *n*-hexadecane ($OD_{660}$ 0.84-1.84), *n*-decane ($OD_{660}$ 0.86-1.55), cyclohexane ($OD_{660}$ 0.52-1.23), paraffin oil ($OD_{660}$ 0.52-2.05), and *n*-hexane ($OD_{660}$ 0.30-0.98) (Figure 3). Like in the plate overlay assay, we observed a better tolerance of the bacterial cells to *n*-hexadecane (log $P_{OW}$ = 9.15), *n*-decane (log $P_{OW}$ 5.98), cyclohexane (log $P_{OW}$ 3.35), or paraffin oil, compared with cells tolerance to *n*-hexane (log $P_{OW}$ 3.86). However, no significant differences were observed between the viability of *P. aeruginosa* $IBB_{Ct8}$ control cells (confluent cell growth) and the viability of the cells grown (24-144 hours) in the presence of these aliphatic hydrocarbons.

*P. aeruginosa* $IBB_{Ct8}$ cells were also able to grow ($OD_{660}$ 0.57-1.39) on the minimal medium with 5% *n*-hexadecane ($OD_{660}$ 1.39), *n*-decane ($OD_{660}$ 0.89), paraffin oil ($OD_{660}$ 0.91), cyclohexane ($OD_{660}$ 0.69), and *n*-hexane ($OD_{660}$ 0.57), as the sole carbon source. Using gravimetric analyses, we monitored the ability of *P. aeruginosa* $IBB_{Ct8}$ cells to degrade 5% aliphatic hydrocarbons. All the tested aliphatic hydrocarbons were well degraded by this bacterium, and the degradation efficiency (between 50-85%) depends on the hydrocarbon type used as the sole carbon source (Figure 3). Higher biodegradation rates (78-85%) were observed for *n*-hexadecane, paraffin oil, and *n*-decane, compared with those obtained for cyclohexane and *n*-hexane (52%, 50%). Furthermore, a direct correlation between bacterial cell growth in the presence of aliphatic hydrocarbons and their degradation potential was observed. *P. aeruginosa* $IBB_{Ct8}$ cells were able to degrade both simple aliphatic hydrocarbons (i.e., *n*-hexadecane, *n*-decane, cyclohexane, *n*-hexane) and also a complex mixture of saturated hydrocarbons (i.e., paraffin oil).




| Hydrocarbon's tolerance, degradation | BG ($OD_{660}$) | | | | DH |
|---|---|---|---|---|---|
| | LBM | | | MM | MM |
| | 24 h | 72 h | 144 h | 144 h | 144 h |
| Control | 0.93 | 1.72 | 2.93 | - | - |
| Cyclohexane | 0.52 | 0.86 | 1.23 | 0.69 | 52% |
| *n*-Hexane | 0.30 | 0.69 | 0.98 | 0.57 | 50% |
| *n*-Decane | 0.86 | 1.43 | 1.55 | 0.89 | 78% |
| *n*-Hexadecane | 0.84 | 1.12 | 1.84 | 1.39 | 85% |
| Paraffin oil | 0.52 | 1.14 | 2.05 | 0.91 | 85% |

| PCR | Primers (-f/-r) | AT (°C) | EFS (bp) | DEF |
|---|---|---|---|---|
| *alkB* | ALK1-f/ALK1-r | 43 | 185 | – |
| *alkM* | ALK2-f/ALK2-r | 43 | 271 | – |
| *alkB1* | ALK3-f/ALK3-r | 43 | 330 | – |
| *alkB* | alkB-f/alkB-r | 60 | 870 | + |
| *alkM1* | alkM-f/alkM-r | 62 | 870 | – |
| *rhlAB* | rhlA-f/rhlB-r | 50 | 216 | + |

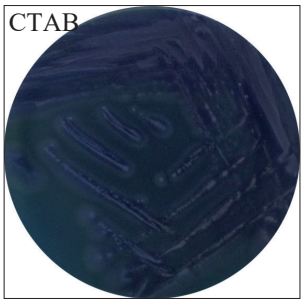
Figure 3. Tolerance of *P. aeruginosa* $IBB_{Ct8}$ to hydrocarbons and their degradation potential
Cells viability (CV), cultures (after 24-144 h incubation) were spotted onto LB agar, control (*1*), cyclohexane (*2*), *n*-hexane (*3*), *n*-decane (*4*), *n*-hexadecane (*5*), paraffin oil (*6*). Bacterial growth (BG), the optical density of the cultures ($OD_{660}$) grown on the LB medium (LBM) and minimal medium (MM). Degradation of hydrocarbons (DH, %). Polymerase chain reaction (PCR) of alkane hydroxylase and rhamnosyltransferase 1 genes, PCR primer (forward -f, reverse -r), annealing temperature (AT, °C), expected fragment size (EFS, bp), detection of the expected fragment (DEF), positive reaction (+), negative reaction (–)

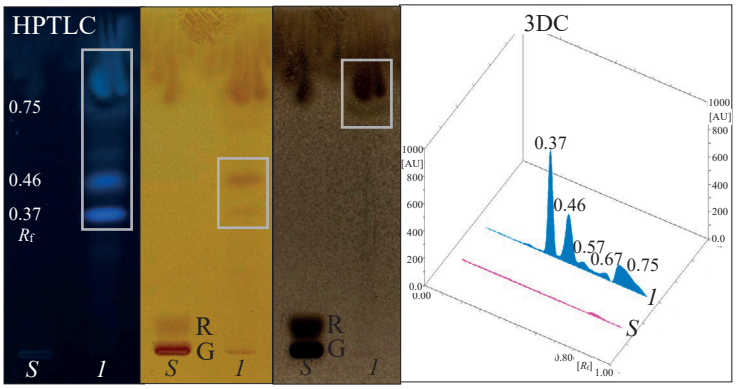
Genomic DNA extracted from *P. aeruginosa* $IBB_{Ct8}$ cells was used as a template for PCR amplification of several known alkane hydroxylases (i.e., *alkB*, *alkM*, *alkB1*, *alkB*; *alkM1*) and rhamnosyltransferase 1 (*rhlAB*) genes responsible for the alkane's degradation and rhamnolipids biosynthesis, respectively. In the DNA extracted from the *P. aeruginosa* $IBB_{Ct8}$ cells was detected the *alkB* (870 bp), and *rhlAB* (216 bp) genes, whereas the *alkB* (185 bp), *alkM* (271 bp), *alkB1* (330 bp), *alkM1* (870 bp) catabolic genes were not detected in this bacterium (Figure 3). Due to their low water solubility, high degree of accumulation in cell membranes, and reactivity, alkane degradation represents a challenge for many bacteria. However, several *P. aeruginosa* strains were previously identified as hydrocarbon-degraders since they have the metabolic capability to use aliphatic hydrocarbons as the sole carbon and energy source (Muriel-Millán et al., 2019; Liu et al., 2022).

**Biosurfactants production**

The biosurfactants production by *P. aeruginosa* $IBB_{Ct8}$ cells was first determined through CTAB methylene blue agar which is a semi-quantitative assay specific for the detection of extracellular glycolipids or other anionic surfactants produced by bacteria (Siegmund and Wagner, 1991). A dark blue halo was observed on this medium for *P. aeruginosa* $IBB_{Ct8}$ colonies (Figure 4). The rhamnolipid surfactants produced by the *P. aeruginosa* $IBB_{Ct8}$ cells were positive for drop-collapse assay and it showed a very good emulsification activity ($E_{24}$ between 70-90%) against cyclohexane, *n*-decane, *n*-hexadecane, paraffin oil, and *n*-hexane (Figure 4). Rhamnolipid produced by other *P. aeruginosa* strain Bs20 showed an appreciable emulsification index with hexadecane, kerosene, diesel, and motor oil ($E_{24}$ between 47-66%) (Abdel-Mawgoud et al., 2009).

The synthesis of rhamnolipid biosurfactants by *P. aeruginosa* $IBB_{Ct8}$ cells was completed by HPTLC analysis of crude surfactants. Under UV light, three fluorescent spots with $R_f$ (retardation factor) 0.37, 0.46, and 0.75 were observed (Figure 4). The first two fractions (with $R_f$ 0.37, 0.47) showed a positive reaction with iodine vapors (light brown spots under visible light), while the third fraction (with $R_f$ 0.75) showed a positive reaction only with orcinol reagent (dark brown spots under visible light) indicating the presence of lipid and sugar moiety in the surfactant molecules.




| Emulsification index ($E_{24}$ %) | |
|---|---|
| Cyclohexane | 78 |
| *n*-Hexane | 70 |
| *n*-Decane | 85 |
| *n*-Hexadecane | 90 |
| Paraffin oil | 75 |

Figure 4. Biosurfactants production by *P. aeruginosa* $IBB_{Ct8}$ CTAB methylene blue agar plate assay. Drop-collapse assay for cell-free culture broths (*1*), positive control (*C+*), negative control (*C-*). Emulsification index ($E_{24}$ %) for cell-free culture broths. HPTLC analysis, sugars standards (*S*), D-glucose (G), L-rhamnose (R), crude biosurfactants extract (*1*), retardation factor ($R_f$), TLC plate was visualized under UV (left side) and visible light (middle, right side), three-dimensional chromatogram (3DC) under UV

On the three-dimensional (3D) chromatogram, we observed five distinct peaks (with $R_f$ 0.37, 0.46, 0.57, 0.67, 0.75) under UV light (Figure 4). Like other bacteria, *P. aeruginosa* strains produce rhamnolipid surfactants, which consist of one or two hydrophilic L-rhamnose molecules and a hydrophobic fatty acid moiety. These glycolipid-type biosurfactants are involved in several functions in the bacterial cells, including in the uptake of poorly soluble substrates, such as aliphatic hydrocarbons enhancing their biodegradation (Bazire and Dufour, 2014; Muriel-Millán et al., 2019).

## CONCLUSIONS

The strain *P. aeruginosa* $IBB_{Ct8}$, which was isolated through the enrichment culture method by a seawater sample possesses in its genome *alkB* (870 bp) and *rhlAB* (216 bp) genes. *P. aeruginosa* $IBB_{Ct8}$ cells showed higher tolerance to *n*-hexadecane, *n*-decane, cyclohexane, or paraffin oil, compared to the *n*-hexane tolerance. Furthermore, *P. aeruginosa* $IBB_{Ct8}$ cells were able to grow on the minimal medium with *n*-hexadecane, *n*-decane, paraffin oil, cyclohexane, and *n*-hexane as the sole carbon source, and their degradation efficiency varied depending on the used hydrocarbon type. Like other bacteria, *P. aeruginosa* $IBB_{Ct8}$ cells produced rhamnolipid surfactants which showed a very good emulsification activity against *n*-hexadecane, *n*-decane, cyclohexane, paraffin oil, and *n*-hexane. This bacterium which showed very good tolerance and degradation efficiency of aliphatic petroleum hydrocarbons has the potential to be used in the bioremediation of petroleum-polluted environments.

## ACKNOWLEDGMENTS

The study was funded by project no. RO1567-IBB05/2022 from the Institute of Biology Bucharest of Romanian Academy. The author is grateful to Ana Dinu for her technical support.

## REFERENCES

Abbasian, F., Lockington, R., Mallavarapu, M. Naidu, R. (2015). A comprehensive review of aliphatic hydrocarbon biodegradation by bacteria. *Appl. Biochem. Biotechnol.*, 176: 670–699.

Abdel-Mawgoud, A. M., Aboulwafa, M. M., Hassouna, N. A. H. (2009). Characterization of rhamnolipid produced by *Pseudomonas aeruginosa* isolate Bs20. *Appl. Biochem. Biotechnol.*, 157: 329–345.

Bazire, A., Dufour, A. (2014). The *Pseudomonas aeruginosa rhlG* and *rhlAB* genes are inversely regulated and RhlG is not required for rhamnolipid synthesis. *BMC Microbiol.* https://doi.org/10.1186/1471-2180-14-160.

Bodour, A. A., Miller-Maier, R. M. (1998). Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *J. Microbiol. Methods*, 32: 273–280.

Eberlein, C., Baumgarten, T., Starke, S., Heipieper, H. J. (2018). Immediate response mechanisms of Gram-negative solvent tolerant bacteria to cope with environmental stress: *cis-trans* isomerization of unsaturated fatty acids and outer membrane vesicle secretion. *Appl. Microbiol. Biotechnol.*, 102: 2583–2593.

Gesheva, V., Stackebrandt, E., Vasileva-Tonkova, E. (2010). Biosurfactant production by halotolerant *Rhodococcus fascians* from Casey Station, Wilkes Land, Antarctica. *Curr. Microbiol.*, 61: 112–117.

Gulati, D., Mehta, S. (2017). Isolation and identification of petrol degrading microorganisms from contaminated soil and comparison of their bioremediation potential. *Int. Res. J. Pharm.*, 8: 34–38.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., Williams, S. T. (1994). Bergey's Manual of Determinative Bacteriology, 9th edition. Williams and Wilkins, Baltimore, USA.

Kohno, T., Sugimoto, Y., Sei, K., Mori, K. (2002). Design of PCR primers and gene probes for general detection of alkane-degrading bacteria. *Microbes Environ.*, 17: 114–121.

Liu, H., Yang, G., Jia, H., Sun, B. (2022). Crude oil degradation by a novel strain *Pseudomonas aeruginosa* AQNU-1 isolated from an oil-contaminated lake wetland. *Processes*, 10. https://doi.org/10.3390/pr10020307.

Márquez-Rocha, F. J., Olmos-Soto, J., Rosano-Hernández, M. C., Muriel-Garcia, M. (2005). Determination of the hydrocarbon-degrading metabolic capabilities of tropical bacterial isolates. *Int. Biodeterior. Biodegrad.*, 55: 17–23.

Medina, G., Juárez, K., Valderrama, B., Soberón-Chávez, G. (2003). Mechanism of *Pseudomonas aeruginosa* RhlR transcriptional regulation of the rhlAB promoter. *J. Bacteriol.*, 185: 5976–5983.

Muriel-Millán, L. F., Rodríguez-Mejía, J. L., Godoy-Lozano, E. E., Rivera-Gómez, N., Gutierrez-Rios, R. M., Morales-Guzmán, D., Trejo-Hernández, M. R., Estradas-Romero, A., Pardo-López, L. (2019). Functional and genomic characterization of a *Pseudomonas aeruginosa* strain isolated from the southwestern Gulf of Mexico reveals an enhanced adaptation for long-chain alkane degradation. *Front. Mar. Sci.*, 6. https://doi.org/10.3389/fmars.2019.00572.

Pini, F., Grossi, C., Nereo, S., Michaud, L., Lo Giudice, A., Bruni, V., Baldi, F., Fani, R. (2007). Molecular and physiological characterisation of psychrotrophic hydrocarbon-degrading bacteria isolated from Terra Nova Bay (Antarctica). *Eur. J. Soil Biol.*, 43: 368–379.

Sambrook, J., Russel, D. (2001). Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Satpute, S. K., Bhawsar, B. D., Dhakephalkar, P. K., Chopade, B. A. (2008). Assessment of different screening methods for selecting biosurfactant producing marine bacteria. *Indian J. Marine Sci*., 37: 243–250.

Shu, Q., Lou, H., Wei, T., Liu, X., Chen, Q. (2021). Contributions of glycolipid biosurfactants and glycolipid modified materials to antimicrobial strategy: a review. *Pharmaceutics*, 13. https://doi.org/10.3390/ pharmaceutics13020227.

Siegmund, I., Wagner, F. (1991). New method for detecting rhamnolipids excreted by *Pseudomonas* species during growth on mineral agar. *Biotechnol. Techn.*, 5: 265–268.

Sikkema, J., de Bont, J. A. M., Poolman, B. (1995). Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.*, 59: 201–222.

Stancu, M. M. (2018a). Bacterial degradation of petroleum and petroleum products. *J. Mol. Microbiol.*, 2(1): 2.

Stancu, M. M. (2018b). Production of some extracellular metabolites by a solvent-tolerant *Pseudomonas aeruginosa* strain. *Waste Biomass Valori.*, 9: 1747–1755.

Stancu, M. M. (2019). Bioremediation of petroleum contaminated environments by *Pseudomonas* species. *J. Biotech. Biores.*, 2(1): JBB.000530.2019.

Stancu, M. M. (2020). Kerosene tolerance in *Achromobacter* and *Pseudomonas* species. *Ann. Microbiol.*, 70(8): 13p. https://doi.org/10.1186/s13213-020-01543-2.

Stancu, M. M., Grifoll, M. (2011). Multidrug resistance in hydrocarbon-tolerant Gram-positive and Gram-negative bacteria. *J. Gen. Appl. Microbiol.*, 57: 1–18.

Torres, S., Pandey, A., Castro, G. R. (2011). Organic solvent adaptation of Gram positive bacteria: applications and biotechnological potentials. *Biotechnol. Adv.*, 29: 442–452.

Whyte, L. G., Greer, C. W., Inniss, W. E. (1996). Assessment of the biodegradation potential of psychrotrophic microorganisms. *Can. J. Microbiol.*, 42: 99–106.