Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XV, 2026
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

HYDROCARBON TOLERANCE AND BIODEGRADATION IN ENVIRONMENTAL *PSEUDOMONAS* STRAINS

Mihaela Marilena STANCU, Simona NEAGU

Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, District 6, Bucharest, P.O. Box 56-53, Romania

Corresponding author email: mihaela.stancu@ibiol.ro

Abstract

Hydrocarbons are widely recognized as the most important class of persistent and toxic environmental pollutants. Moreover, bacteria from the genus Pseudomonas play a key role in the biodegradation of these compounds, and their remarkable metabolic versatility enables them to adapt to a wide range of contaminated environments, enhancing their effectiveness in pollutant removal. Although many species in this genus have already been identified, new Pseudomonas strains with significant relevance for environmental bioremediation continue to be reported each year. This study investigated hydrocarbon-tolerant and hydrocarbon-degrading bacteria in sediment samples from the Danube Delta collected during summer and autumn. Additionally, the ability of four Pseudomonas strains, individually and as a consortium, to tolerate and degrade paraffin (a mixture of saturated petroleum hydrocarbons) was evaluated. The tested strains demonstrated promising potential for paraffin biodegradation, particularly when applied as a consortium, likely due to functional complementarity and enhanced metabolic interactions. These findings provide valuable insights for environmental applications, including land reclamation and ecological engineering, and contribute to the development of effective strategies to mitigate petroleum hydrocarbon pollution and restore contaminated ecosystems.

Key words: *biodegradation, paraffin, Pseudomonas, tolerance.*

INTRODUCTION

Pollution caused by petroleum and petroleum-based products, including paraffinic oils, is widely recognized as a major environmental issue due to the widespread use and extensive distribution of hydrocarbon-based materials in natural ecosystems. As industrial activities and urbanization intensify, hydrocarbon contamination in water bodies has become increasingly problematic, contributing substantially to the degradation of aquatic habitats. Although numerous strategies and technologies are currently employed to prevent and control global contamination by petroleum and petroleum-based products, the increasing frequency of accidents in aquatic environments continues to make hydrocarbon pollution a significant challenge in the coming decades. In this context, biodegradation is considered a key process, as it represents one of the primary and most cost-effective processes for removing hydrocarbons from polluted environments (Das & Chandran, 2011; Xu et al., 2018; Xu et al., 2022; Duarte et al., 2024; Al Haj Chehadeh et al., 2025; Samsuria et al., 2025).

Petroleum and petroleum-based products are highly complex mixtures composed of thousands of individual compounds, which are generally grouped into four classes based on their structural characteristics and physicochemical properties: saturated (aliphatic) hydrocarbons, aromatic hydrocarbons, resins, and asphaltenes (Das & Chandran, 2011; Pandolfo et al., 2023). Each class is characterized by distinct yet interrelated physicochemical properties – particularly toxicity, volatility, solubility, molecular weight, and biodegradability – which collectively influence its environmental fate, transport, and transformation in aquatic systems. When petroleum and its derivatives enter these systems, they act as environmental stressors, with toxic effects extending across trophic levels, affecting phytoplankton, zooplankton, fish, birds, mammals, and benthic communities. Beyond immediate ecological disruption, such releases alter biodiversity, degrade water quality, and generate long-term socio-environmental consequences for surrounding human populations (Perhar &

Arhonditsis, 2014; Duarte et al., 2024; Sharma et al., 2024; Samsuria et al., 2025).

In aquatic environments, the lightest and most volatile hydrocarbon fractions tend to volatilize rapidly into the atmosphere. In contrast, amphipathic and hydrophilic components dissolve or disperse in the water column, while more lipophilic fractions preferentially associate with suspended particulate matter and organic colloids (Pandolfo et al., 2023). These hydrophobic constituents further bind to particulates and sediments, promoting their accumulation and facilitating exposure in benthic species (Perhar & Arhonditsis, 2014; Pandolfo et al., 2023). The accumulation of hydrocarbons in sedimentary matrices can alter microbial community structure, suppress the metabolic activity of sensitive sediment bacteria, and disrupt key biogeochemical processes such as nutrient cycling and organic matter degradation. These sediments may also harbor bacterial taxa capable of degrading hydrocarbons, thereby contributing to natural attenuation in contaminated benthic environments. At the same time, hydrocarbons can eliminate or inhibit susceptible microbial taxa, thereby altering key ecosystem functions. The selective pressure exerted by these compounds often reduces microbial diversity and shifts community composition toward bacterial populations that use hydrocarbons as carbon and energy sources. Given this dual role of hydrocarbons in both constraining susceptible microorganisms and selecting for specialized microbial communities, the characterization of bacteria capable of hydrocarbon degradation is essential for assessing natural attenuation potential in aquatic sediments (Das & Chandran, 2011; Pandolfo et al., 2023; Al Haj Chehadeh et al., 2025; Samsuria et al., 2025).

This study investigated hydrocarbon-tolerant and hydrocarbon-degrading bacteria in sediment samples from the Danube Delta collected during summer and autumn. Additionally, the tolerance and biodegradation capacity of four *Pseudomonas* strains, tested individually and as a consortium, were evaluated using paraffin, a mixture of saturated petroleum hydrocarbons characterized by high chemical stability and low biodegradability.

MATERIALS AND METHODS

Quantification of bacteria in sediments

Sediment samples from the Danube Delta (Roșu, Roșuleț, and Puiu lakes, Tulcea County, Romania), part of the southern lacustrine complex within the Danube Delta Biosphere Reserve, were used for the development of enrichment cultures. The experimental setup was based on a modified minimal medium (Stancu, 2023), supplemented with petroleum (5%, v/v) as the sole carbon and energy source. Cultures were maintained at 30°C under constant agitation (200 rpm) for 21 days to promote the growth of hydrocarbon-adapted microorganisms. The abundance of hydrocarbon-tolerant and hydrocarbon-degrading bacteria was determined using two culture-dependent quantification methods: the most probable number (MPN) and colony-forming unit (CFU) counts (Stancu, 2025).

Paraffin tolerance and biodegradation by *Pseudomonas* strains and consortium

Four *Pseudomonas* strains (IBBDt2, IBBDt4.2, IBBDt1, and IBBDt8), previously isolated and identified (Stancu, 2023), were cultured in LB medium at 30°C under shaking conditions (200 rpm) for 24 h. Subsequently, the antagonistic interactions among the four strains were assessed using the cross-streak method (Hossain, 2024). In this assay, one strain was initially streaked onto LB agar and incubated at 30°C for 24 h, after which the remaining strains were spot-inoculated in proximity to the primary streak.

Tolerance to paraffin and biodegradation potential of the four bacterial strains, both individually and as a consortium (CnP), were further evaluated. Standardized overnight cultures (OD_{660} = 0.08) were inoculated into fresh LB and MM media supplemented with paraffin (5%, v/v) and incubated at 30°C under shaking conditions (200 rpm) for 7 days. Uninoculated MM supplemented with paraffin (5%, v/v) served as an abiotic control.

Bacterial growth was assessed through optical density measurements at 660 nm (OD_{660}), using a SPECORD 200 UV-Vis spectrophotometer (Analytik Jena). Biomass accumulation was further estimated by determining dry cell weight. Bacterial viability was evaluated by

spotting paraffin-exposed cultures onto LB and MM agar media, both in the presence and absence of a paraffin overlay. To assess growth dynamics, paraffin-exposed cultures were transferred into fresh LB medium and incubated at 30°C under controlled conditions. Changes in optical density (OD_{660}) were monitored at hourly intervals over a 24 h period using a SpectraMax ABS Plus microplate reader (Molecular Devices).

Paraffin biodegradation was evaluated using a combination of complementary approaches, including observation of paraffin film fragmentation, gravimetric determination of residual paraffin recovered from cell-free supernatants after chloroform extraction, and high-performance thin-layer chromatography (HPTLC), following a previously described method (Stancu, 2023) with modifications for paraffin analysis. Chromatographic analyses were performed using a CAMAG TLC system, and 3D profiles were processed using visionCATS software (CAMAG). Samples were applied to silica gel plates and developed in a pre-saturated chamber with an n-hexane–ethyl acetate (92:8, v/v) mobile phase. Plates were then treated with iodine vapors and analyzed under visible light (Vis, 500 nm).

Biosurfactant production was assessed using several complementary assays, including the hydrocarbon overlay agar plate method, drop-collapse test, CTAB assay, and the emulsification index (E_{24}), as previously described (Satpute et al., 2008). In addition, biosurfactant yield was quantified gravimetrically after extraction from cell-free culture supernatants using a chloroform–methanol mixture (2:1, v/v), and subsequent solvent evaporation (Gesheva et al., 2010).

The presence of alkane-degrading genes (*alkB* and *alkM*) and the rhamnosyltransferase gene (*rhlAB*) was investigated by polymerase chain reaction (PCR). Genomic DNA was isolated using the PureLink Genomic DNA Kit (Invitrogen). Amplification reactions were performed in a Mastercycler Pro S thermocycler (Eppendorf) with gene-specific primers (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). PCR amplification included an initial denaturation at 94°C for 10 min, followed by 35 cycles (94°C for 1 min, 50-62°C for 30 s, and 72°C for 2 min) and a final extension at 72°C for 10 min. Amplified fragments were resolved by electrophoresis on 1.5% (w/v) agarose gels (Sambrook & Russell, 2001) and visualized using SYBR Safe DNA gel stain (Invitrogen).

Changes in genomic fingerprinting profiles were analyzed by repetitive extragenic palindromic PCR (REP-PCR) using the $(GTG)_5$ primer (Versalovic et al., 1994) and GoTaq G2 Flexi DNA Polymerase (Promega). Amplification was performed in a Mastercycler Pro S thermocycler (Eppendorf).

PCR conditions included an initial denaturation at 94°C for 10 min, followed by 35 cycles (94°C for 1 min, 52°C for 1 min, and 65°C for 8 min) and a final extension at 65°C for 16 min. The resulting fragments were separated on 2.0% (w/v) agarose gels and visualized as described above.

Reagents used in this study were obtained from Merck and Sigma-Aldrich.

RESULTS AND DISCUSSIONS

Quantification of bacteria in sediments

Petroleum and petroleum-based products frequently contaminate aquatic ecosystems, entering through industrial activities, shipping activities, accidental spills, and urban wastewater. In the Danube Delta, a protected wetland of high ecological value, hydrocarbon accumulation in sediments poses a long-term threat to biodiversity and ecosystem processes.

The abundance of hydrocarbon-tolerant and hydrocarbon-degrading bacteria in sediment samples collected in summer (July) and autumn (September) from the Danube Delta was assessed after enrichment in petroleum-amended minimal medium (MM). Bacterial populations were quantified using MPN and CFU approaches (Figure 1), providing insights into the sediment microbial community's natural attenuation potential and seasonal variations. Regardless of the quantification method, hydrocarbon-tolerant bacteria were consistently present at higher levels (10^4-10^6 cells mL^{-1}, 10^3-10^6 CFU mL^{-1}) than hydrocarbon-degrading bacteria (10^2-10^3 cells mL^{-1}, 10^1-10^3 CFU mL^{-1}). Seasonal differences were also observed for both groups. Sediment

samples collected in July showed higher levels of hydrocarbon-tolerant bacteria (10^5-10^6 cells mL^{-1}, 10^4-10^6 CFU mL^{-1}) than those collected in September (10^4-10^5 cells mL^{-1}, 10^3-10^4 CFU mL^{-1}). Similarly, hydrocarbon-degrading bacteria were more abundant in July samples (10^3 cells mL^{-1}, 10^3 CFU mL^{-1}) than in September samples (10^2 cells mL^{-1}, 10^1 CFU mL^{-1}). These patterns likely reflect seasonal temperature effects on bacterial growth and hydrocarbon metabolism. The predominance of hydrocarbon-tolerant heterotrophs over specialized degraders aligns with previous studies, where metabolically versatile degraders represented a smaller fraction of the microbial community in hydrocarbon-exposed sediments (Xu et al., 2022

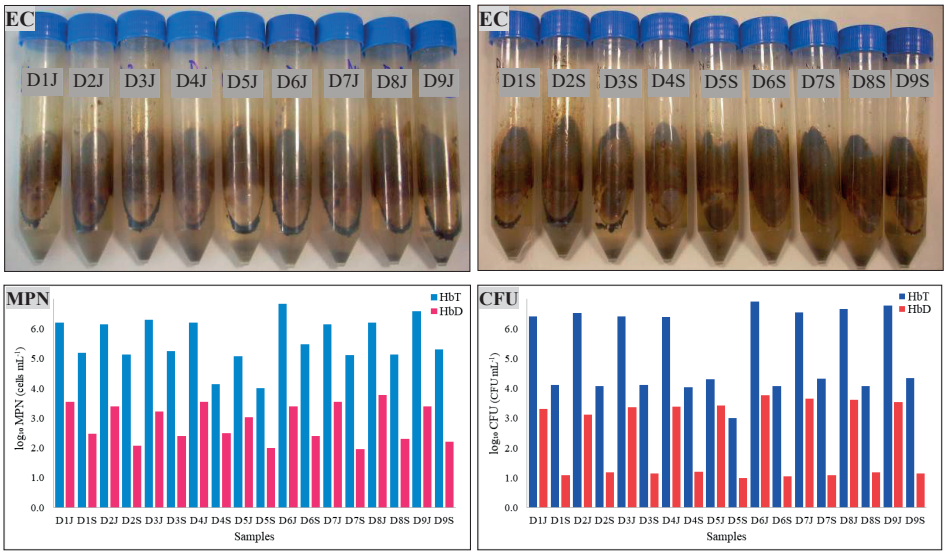


Figure 1. Quantification of bacteria in sediments
Sediment samples collected in July (D1J-D9J) and September (D1S-D9S) were analyzed after enrichment (EC) for hydrocarbon-tolerant (HbT) and hydrocarbon-degrading (HbD) bacteria using MPN and CFU methods (log_{10} scale)

Paraffin tolerance and biodegradation by *Pseudomonas* strains and consortium

The *Pseudomonas* strains IBBDt2, IBBDt4.2, IBBDt1, and IBBDt8, previously identified as *P. aeruginosa*, *P. nitroreducens*, *P. aeruginosa*, and *P. multiresinivorans*, respectively (Stancu, 2023), were evaluated for paraffin tolerance and biodegradation, both individually and in consortium. *Pseudomonas* species produce secondary metabolites, such as pyocyanin, pyoverdine, and biosurfactants, which affect microbial interactions through redox reactions, iron chelation, and membrane destabilization (Chong & Li, 2017; Butaitė et al., 2018; Abdelaziz et al., 2023). Consequently, strain compatibility was assessed before the experiments, given the selected bacteria's capacity to produce metabolites, such as pyocyanin and/or pyoverdine, and biosurfactants (Stancu, 2023). No antagonistic interactions were detected among four *Pseudomonas* strains (Figure 2), consistent with reports that metabolite production does not necessarily induce inhibition under all growth conditions.

Bacterial growth. All four *Pseudomonas* strains IBBDt2, IBBDt4.2, IBBDt1, and IBBDt8, grown individually and as a consortium in LB broth supplemented with 5% paraffin, presented high tolerance, as indicated by optical density at 660 nm (OD_{660} = 1.06-1.45) and dry biomass measurements (3.54-5.17 g L^{-1}) (Table 1). In addition, both the individual strains and the consortium grew in MM broth supplemented with 5% paraffin as the sole carbon and energy source (OD_{660} = 0.63-0.87, dry biomass = 1.22-3.34 g L^{-1}).

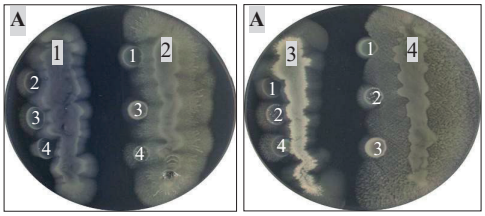


Figure 2. Antagonistic interactions among *Pseudomonas* strains
Antagonistic activity (AA) of strains IBBDt2 (1), IBBDt4.2 (2), IBBDt1 (3), and IBBDt8 (4), evaluated by testing each strain against the other three on LB agar

In tolerance (LB) and biodegradation (MM) experiments, the *Pseudomonas* consortium exhibited enhanced growth in the presence of paraffin (OD_{660} = 1.45 and 0.87, respectively) compared to the individual strains (OD_{660} = 1.06–1.36 and 0.63–0.83, respectively). Although differences in OD and dry biomass values were observed among the experimental variants, viability assays performed after 7 days of growth in LB or MM with 5% paraffin revealed confluent growth of all cultures on LB agar, with or without paraffin overlay (Figure 3). In contrast, reduced viability was observed on MM agar overlaid with paraffin, suggesting metabolic stress under nutrient limitation (Márquez-Rocha et al., 2005). Although reduced viability was observed under nutrient-limited conditions, the individual strains and the consortium maintained growth in the presence of paraffin, suggesting adaptability to hydrocarbon substrates, likely through alkane catabolic pathways (Whyte et al., 1996; Das & Chandran, 2011).

Pseudomonas strains cultivated for 7 days as individual cultures or as a consortium in LB and MM broths supplemented with 5% paraffin, and subsequently reinoculated into fresh LB broth, showed a lag phase of approximately 3-4 h (Figure 3). In all cases, this initial adaptation period was followed by an exponential growth phase occurring between 4 and 18 h. After approximately 15-19 h of incubation, the cultures entered a stationary (plateau) phase. The onset and duration of these phases varied across individual strains and the consortium. This observation was further supported by the determined growth rates and generation times (Table 1). Cultures previously grown for 7 days in LB broth with 5% paraffin showed growth rates (μ) ranging from 0.61 to 0.87 h^{-1} and generation times (g) between 0.80 and 1.13 h, whereas cultures grown in MM broth with 5% paraffin had lower growth rates (μ = 0.55-0.72 h^{-1}) and longer generation times (g = 0.96-1.25 h) when transferred to fresh LB. Cultivation under nutrient-rich conditions favors faster adaptation and enhanced growth kinetics, while minimal media impose metabolic constraints, reducing growth rates and prolonging generation times (Hall et al., 2014).

Table 1. Paraffin tolerance and biodegradation by *Pseudomonas* strains and consortium

Parameters	Individual strains				Consortium
	IBBDt2	IBBDt4.2	IBBDt1	IBBDt8	CnP
Growth quantification					
Tolerance assay					
Optical density (OD_{660})	1.16	1.26	1.36	1.06	1.45
Dry cell weight (g L^{-1})	4.87	3.69	4.37	3.54	5.17
Bacterial viability	CG	CG	CG	CG	CG
Biodegradation assay					
Optical density (OD_{660})	0.71	0.63	0.83	0.79	0.87
Dry cell weight (g L^{-1})	2.29	2.01	2.53	1.22	3.34
Bacterial viability	CG	CG	CG	CG	CG
Growth kinetics					
Tolerance assay					
Specific growth rate (μ, h^{-1})	0.87	0.82	0.61	0.82	0.69
Generation time (g, h)	0.80	0.84	1.13	0.84	1.00
Biodegradation assay					
Specific growth rate (μ, h^{-1})	0.56	0.62	0.55	0.57	0.72
Generation time (g, h)	1.24	1.12	1.25	1.21	0.96
Paraffin biodegradation					
Paraffin film fragmentation	+	+	+	+	+
Biodegradation (%)	75.64	69.25	77.70	60.88	85.45
PCR *alkB* gene (870 bp)	+	+	+	+	+

Parameters	Individual strains				Consortium
	IBBDt2	IBBDt4.2	IBBDt1	IBBDt8	CnP
PCR *alkM* gene (870 bp)	+	+	–	+	+
Biosurfactant production					
Hydrocarbon overlay agar	+	+	+	+	+
Glycolipid production (CTAB)	+	+	+	+	+
Drop-collapse assay	+	+	+	+	+
Emulsification index (E_{24}, %)	100	50	100	30	100
Biosurfactant quantification (g L^{-1})	0.92	0.31	1.22	0.41	1.89
PCR *rhlAB* genes (216 bp)	+	–	+	–	–

Confluent growth (CG). Positive reaction (+); negative reaction (–).

Paraffin biodegradation. *Pseudomonas* strains IBBDt2, IBBDt4.2, IBBDt1, and IBBDt8, cultivated individually and as a consortium in MM broth supplemented with 5% paraffin, disrupted the paraffin film, indicating emulsification and degradation (Table 1). Gravimetric analyses confirmed the capacity of tested bacteria to biodegrade paraffin, with degradation efficiencies ranging from 60.88% to 85.45%. The highest biodegradation efficiency was achieved for the *Pseudomonas* consortium (85.45%), exceeding that noticed for the individual strains (60.88-77.70%), suggesting a synergistic interaction within the consortium that enhances paraffin biodegradation. Among the individual strains, IBBDt1, IBBDt2, and IBBDt4.2 exhibited comparatively higher biodegradation efficiencies (77.70%, 75.64%, and 69.25%, respectively), whereas strain IBBDt8 showed the lowest efficiency (60.88%). Previous studies have reported that bacterial consortia display superior hydrocarbon-degrading performance compared to pure cultures due to complementary metabolic pathways, synergistic enzymatic activities, and biosurfactant production, which enhance hydrocarbon bioavailability and overall biodegradation efficiency (Das & Chandran, 2011; Varjani, 2017; Xu et al., 2018).

Following the gravimetric evaluation of paraffin degradation, residual paraffin samples were subjected to HPTLC analyses. These analyses indicated the presence of a single spot migrating close to the solvent front, with retardation factor (R_f) values ranging from 0.82 to 0.86 (Figure 3). Three-dimensional chromatographic (3DC) profiles confirmed differences in peak intensity among the samples. In the abiotic control, the R_f value was 0.84. Similar R_f values (0.82-0.83) were recorded for extracts obtained from *Pseudomonas* strains IBBDt2, IBBDt1, IBBDt8, and the consortium, whereas the extract from strain IBBDt4.2 showed a slightly higher R_f value (0.86). For this strain, the detected spot was of low intensity and markedly smaller than the other chromatographic spots. Although the *Pseudomonas* consortium and strains IBBDt1 and IBBDt2 presented higher gravimetric biodegradation efficiencies, their HPTLC profiles showed minor differences compared to the abiotic control, consistent with the semi-quantitative nature of HPTLC. In contrast, strain IBBDt4.2, despite its moderate biodegradation efficiency (69.25%), displayed a reduced spot intensity, suggesting preferential degradation of the paraffin fraction corresponding to this chromatographic spot. These results indicate that different strains may target distinct paraffin fractions. Genomic DNA extracted from paraffin-exposed cultures was used for PCR detection of the *alkB* and *alkM* genes, which encode alkane hydroxylases responsible for the initial step of alkane oxidation (Table 1, Figure 4). The *alkB* gene (≈870 bp) was detected in strains IBBDt2, IBBDt1, IBBDt8, and in the consortium, whereas strain IBBDt4.2 produced a slightly smaller amplicon (~850 bp). The *alkM* gene (≈870 bp) was present in strains IBBDt2, IBBDt4.2, and IBBDt8, while only weak amplification was observed in the consortium, and no amplification was detected in strain IBBDt1. Together, these results confirm the alkane-degrading potential of the individual *Pseudomonas* strains and the consortium, in agreement with previous studies reporting a strong correlation between the presence of *alkB*/*alkM* genes and efficient alkane biodegradation in hydrocarbon-degrading bacteria (Whyte et al., 1996; Márquez-Rocha et al., 2005; Das & Chandran, 2011; Pandolfo et al., 2023).

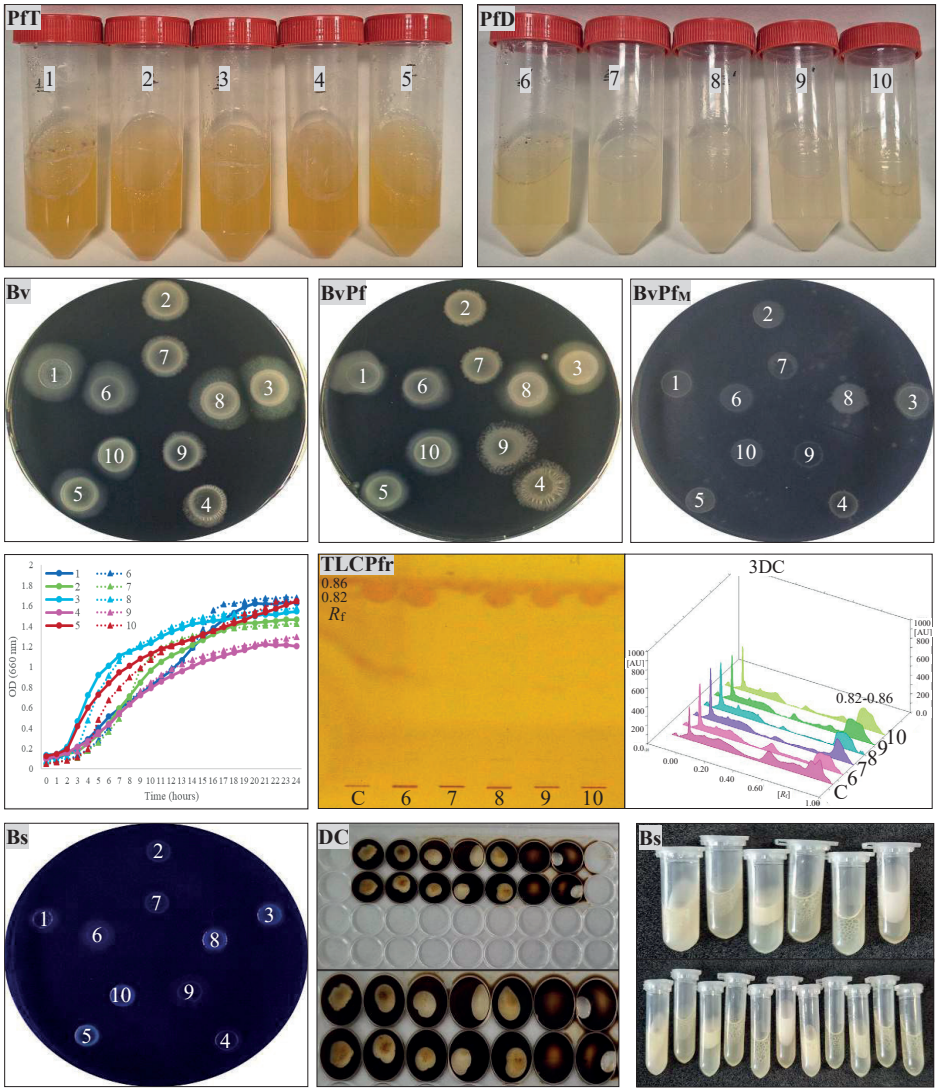


Figure 3. Paraffin tolerance and biodegradation by *Pseudomonas* strains and consortium
Paraffin tolerance (PfT, 1–5) and biodegradation assays (PfD, 6–10) performed for strains IBBDt2 (1, 6), IBBDt4.2 (2, 7), IBBDt1 (3, 8), IBBDt8 (4, 9), and the consortium (CnP, 5, 10). Bacterial viability on LB agar (Bv) and LB or MM agar overlaid with paraffin (BvPf, BvPf$_M$). Growth curves of the tested strains and consortium (Gc). HPTLC analysis of residual paraffin (TLCPfr, R_f 0.82–0.86) compared with abiotic control (C), including TLC plate and 3D chromatogram (VIS). Biosurfactant production on CTAB agar (Bs); drop-collapse assay (DC); and emulsification assay (Es).

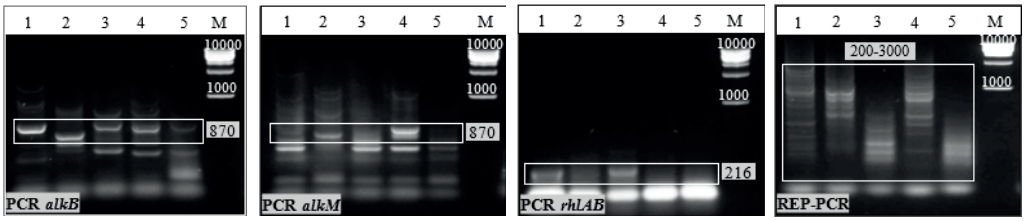


Figure 4. Molecular characterization of *Pseudomonas* strains and consortium
PCR detection of *alkB* (870 bp), *alkM* (870 bp), and *rhlAB* (216 bp) genes using DNA from strains IBBDt2 (1), IBBDt4.2 (2), IBBDt1 (3), IBBDt8 (4), and the consortium (CnP, 5); 1 kb DNA ladder (M). REP-PCR fingerprinting profiles of the same strains (1–4) and consortium (5).

Biosurfactant production. *Pseudomonas* strains IBBDt2, IBBDt4.2, IBBDt1, and IBBDt8, cultivated individually and as a consortium in the presence of 5% paraffin, presented biosurfactant production, as evidenced by positive results in the hydrocarbon overlay and drop-collapse assays (Table 1, Figure 3). All tested strains, as well as the consortium, produced a blue halo on CTAB agar when paraffin-grown cultures were spotted, indicating the synthesis of glycolipid or other anionic biosurfactants. The produced biosurfactants presented strong emulsification activity, with emulsification index (E_{24}) values ranging from 30% to 100% (Table 1). Maximum E_{24} values (100%) were observed for the *Pseudomonas* strains IBBDt1 and IBBDt2, as well as for the consortium containing all four strains, followed by strains IBBDt4.2 (50%) and IBBDt8 (30%). Biosurfactant production by the individual *Pseudomonas* strains and the consortium ranged from 0.31 to 1.89 g L^{-1}. The *Pseudomonas* consortium presented the highest biosurfactant yield (1.89 g L^{-1}), followed by strains IBBDt1 (1.22 g L^{-1}) and IBBDt2 (0.92 g L^{-1}), whereas lower yields were recorded for strains IBBDt8 (0.41 g L^{-1}) and IBBDt4.2 (0.31 g L^{-1}). This enhanced biosurfactant production (1.89 g L^{-1}) by the consortium could be attributed to synergistic interactions among its constituent strains, which likely contribute to their superior paraffin biodegradation efficiency (85.45%). *Pseudomonas* spp. efficiently utilizes petroleum and petroleum-based products as carbon and energy sources and produce biosurfactants that facilitate hydrocarbon emulsification and biodegradation in contaminated environments (Chong & Li, 2017; Cui & Dörmann, 2025).

PCR amplification targeting the *rhlAB* genes encoding rhamnosyltransferase I detected the expected 216 bp fragment exclusively in the paraffin-exposed individual strains IBBDt2 and IBBDt1, both identified as *P. aeruginosa* (Table 1, Figure 4). These genes encode key enzymes involved in the biosynthesis of mono- and di-rhamnolipids, glycolipid biosurfactants that facilitate hydrocarbon solubilization and uptake. Previous studies indicate that *rhlAB* genes are predominantly associated with *P. aeruginosa* and play a central role in rhamnolipid production (Medina et al., 2003; Chong & Li, 2017; Wongsirichot & Winterburn, 2025). In contrast, no amplification products were obtained from *P. nitroreducens* IBBDt4.2, *P. multiresinivorans* IBBDt8, or from the consortium, despite the presence of the two *P. aeruginosa* strains. The absence of detectable *rhlAB* amplification in the consortium may be attributed to a lower relative abundance of *P. aeruginosa* genomic DNA, dilution effects during DNA extraction, or preferential amplification of dominant genomic templates in mixed bacterial communities, as reported in PCR-based analyses of complex microbial communities (van Dorst et al., 2014).

REP-PCR fingerprinting revealed distinct banding patterns (ranging from 200 to 3000 bp) for the four tested *Pseudomonas* strains when cultivated individually, confirming their genomic heterogeneity (Figure 4). In contrast, the consortium displayed a simplified REP-PCR profile, characterized by fewer bands predominantly between 200 and 400 bp. Several bands observed in the consortium overlapped with those detected in strain IBBDt1. The reduced complexity of the consortium fingerprint may indicate a shift in

population structure during incubation in 5% paraffin, possibly reflecting the numerical predominance of IBBDt1 under these conditions. Despite this apparent structural simplification, the consortium maintained superior paraffin biodegradation efficiency compared to the individual strains.

Our results demonstrate the promising potential of the tested *Pseudomonas* strains for paraffin biodegradation, particularly when combined in a consortium, likely due to functional complementarity and enhanced metabolic interactions. Collectively, these findings highlight their potential applicability in environmental biotechnology, including land reclamation and ecological engineering strategies aimed at mitigating petroleum hydrocarbon pollution and restoring contaminated ecosystems.

CONCLUSIONS

Hydrocarbon-tolerant and hydrocarbon-degrading bacteria exhibited higher abundances during summer, with hydrocarbon-tolerant bacteria consistently dominating, indicating a clear seasonal influence on their distribution.

The tested *Pseudomonas* strains, both individually and as a consortium, demonstrated good ability to tolerate and degrade paraffin, indicating high metabolic versatility and suggesting their potential applicability in the bioremediation of sediments contaminated with saturated petroleum hydrocarbons.

Overall, this study enhances the understanding of the functional diversity and ecological role of hydrocarbon-degrading bacteria in natural sediments and provides a scientific basis for the development of environmentally friendly monitoring and bioremediation strategies in sensitive ecosystems such as the Danube Delta.

ACKNOWLEDGEMENTS

The study was funded by project no. RO1567-IBB05/2026 from the Institute of Biology Bucharest of Romanian Academy.

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