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دراسة مقارنة لإنتاجية وفعالية البايوسيرفاكتنت بين بكتيريا *Pseudomonas aeroginosa* و*Pseudomonas putida* المعزولة من التربة الملوثة بالنفط في العراق

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الملخص

تقدم هذه الدراسة تحليلاً مقارناً لاستخلاص العوامل الفعّالة سطحياً من نوعين بكتيريين ينتميان لجنس الزائفة، والمعزولين من تربة ملوثة بالنفط في الرمادي بالعراق. اعتمد البحث على التشخيص الجزيئي باستخدام جين(16SrRNA) ومطابقة النتائج مع بيانات NCBI العزلة (S43) كانت الزائفة الزنجارية والعزلة (S54) كانت الزائفة البيوتيدية.

برزت النتائج قدرة كل من النوعين المشخصة على إنتاج مركبات فعالة سطحياً ذات خصائص كيميائية ووظيفية متميزة.وكشف التحليل الطيفي بالأشعة تحت الحمراء عن تنوع كيميائي في المستخلصات المنتجة بينما اكد تحليل كروماتوغرافيا الغاز-مطياف الكتلة احتواءها على مركبات دهنية وببتيدية تدعم فعاليتها السطحية, اظهر البايوسيرفكتنت الناتج من بكتريا الزائفة الزنجارية خصائص فعالية سطحية متفوقة, تمثلت في انخفاض تركيز المذيلات الحرج وارتفاع مؤشر الاستحلاب, مما ارتبط ارتباطا مباشرا بكفاءتها المحسنة في تحليل الهيدروكاربونات. تقدم هذه النتائج رؤى قيمة لاختيار انواع السيدوموناس المناسبة لتطبيقات المعالجة الحيوية المحددة بناء على طبيعة الملوثات والظروف البيئية .

الكلمات المفتاحية: البايوسيرفكتنت ,الزائفة الزنجارية ,الزائفة البيوتيدية, تحلل الهيدروكاربونات,التشخيص الجزيئي ,GC-MS,FTIR, المعالجة الحيوية

Comparative Study of Biosurfactant Production and Activity Between *Pseudomonas aeruginosa* and *Pseudomonas putida* Isolated from Oil-Contaminated Soils in Iraq

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Abstract

We here for the first time report a comprehensive study on comparative biosurfactant production and activities by two Pseudomonas isolated from crude oil contaminated soil in Ramadi, Iraq. Sequencing of the 16S rRNA gene confirmed that R23 was Pseudomonas aeruginosa (100 % similarity) and S54 was Pseudomonas putida (98 % similarity) in comparison to reference strains. FTIR absorbance and amide I signal at approximately 3415-3437 (O-H/N-H stretching) and 2922-2852 cm^{-1} (aliphatic C-H stretching) of the FTIR absorbed at wave numbers 1635-1645 cm^{-1} of amides II made at supramolecular level by PCM with a change solvents suggested, thus resulting compounds were amphiphilic in nature. A GC-MS characterization of the two species was performed. 3-hydroxydecanoic acid (18.5%), decanoic acid (12.4%) and 3-hydroxydodecanoic acids (9.8%)). Pseudomonas putida S54, on the other hand, was richer in composition and possessed decanoic acid (8.9%) and dodecanoic acid (10.2%), along with some aromatic compounds such as catechol (14.3%) and protocatechuic acid (2.5%). The surface active performances of biosurfactants from P.aeruginosa have been found to higher than the strains used in this study (Mahesh et al. 2009) with strikingly reduced critical micelle concentration and higher emulsification index, traits which are largely responsible for their enhanced crude oil degradation. The kinetics of the process must be characterized in order to choose Pseudomonas strains for bioremediation according to pollution and conditions.

Keywords: Biosurfactants, *Pseudomonas aeruginosa*, *Pseudomonas putida*, Hydrocarbon degradation, Molecular characterization, FTIR, GC-MS, Bioremediation.

1. Introduction

Hydrocarbons contain a m? xture? The aliphatic and aromatic hydrocarbon with C35 as the highest carbon number is one of the known for bioremediation of petroleum hydrocarbons, that is a challenge to environment particularly in oil producing countries such as Iraq where soil pollution by hydrocarbons significantly effects ecosystem balance and crop yields. Bacterial bioremediation practices also followed that pattern hence, the resulting to shorter half-lives are noticed when the surfactant producing bacteria is amended along with hydrocarbons [1], which proves to be more environmentally appealing in hydrocarbon degradation from contaminated soil. The most commonly

investigated microorganisms, on the basis of metabolic diversity and efficient role in biosurfactant production are primarily Pseudomonas species (2)

In Pseudomonas, two isolates (P. aeruginosa and P. putida) are already defined as they exhibit differential characteristics in terms of biosurfactant production as well hydrocarbon degradation rates. CcHOW-utilising P. aeruginosa is a particularly interesting, partially because it has been shown to synthesise Rhamnolipids (one of the most potent category of biosurfactants with emulsifying capacity and hydrocarbon degradation [3]). (In contrast, P. putida is metabolically more versatile; it is the bacterium responsible for the production of other biosurfactants such as putisolvin and a variety of glycolipids and hydrogenylases capable of degrading aromatic hydrocarbons [4].Although multiple investigations have focused on specific Pseudomonas species, thorough comparative examinations under standardized conditions remain scarce. Comprehending the unique benefits and constraints of each species is vital for enhancing bioremediation approaches across various environmental contexts [5]. This research aims to fill this informational void by conducting a methodical evaluation of biosurfactant production and functionality between P. aeruginosa and P. putida obtained from Iraqi oil-polluted soils, including molecular identification, structural examination, and performance assessment of their biosurfactants.

2. Materials and Methods

2.1 Sample Collection and Bacterial Isolation

Soil specimens were systematically gathered from seven distinct petroleum-impacted locations within the Ramadi region of Iraq. Collection procedures maintained strict aseptic conditions, utilizing sterilized spatulas to obtain topsoil layers extending to approximately 10 cm depth. The acquired samples were immediately transferred to pre-sterilized polyethylene containers with secure sealing to preserve sample integrity. Transportation to laboratory facilities involved continuous refrigeration on ice packs, with subsequent storage at 4°C to maintain microbial viability until analytical processing commenced [6].

Microbial isolation procedures employed selective enrichment strategies using Mineral Salt Medium (MSM) formulations, where crude petroleum served as the exclusive carbon substrate. The enrichment process involved three sequential cultivation cycles to promote the proliferation of hydrocarbon-adapted bacterial

communities. Subsequent purification of bacterial isolates was achieved through systematic streak-plating methodology on nutrient-supplemented agar surfaces, ultimately yielding axenic cultures for further investigation [7].

2.2 Molecular Identification

Bacterial genomic DNA isolation was performed employing the TransGen Biotech extraction kit following established protocols. Amplification of the 16S rRNA gene fragments was carried out through polymerase chain reaction (PCR) utilizing the universal primer set U16SRT-F (5′-ACTCCTACGGGAGGCAGCAGT-3′) and U16SRT-R (5′-TATTACCGCGGCTGCTGGC-3′), following established molecular methodologies [8]. The resulting amplification products underwent commercial sequencing, and subsequent microbial classification was achieved through comparative analysis against the NCBI GenBank database using the BLASTn algorithm. A sequence similarity cutoff of 98% or higher was implemented as the criterion for reliable species-level identification, in accordance with standard taxonomic practices [9].

2.3 Biosurfactant Production and Extraction

The chosen bacterial strains were propagated in mineral salts medium (MSM) amended with 1% (v/v) paraffin oil as the primary carbon substrate. Cultivation occurred at 30°C with continuous agitation at 150 rpm over a 72-hour period. Following incubation, the broth was clarified by centrifugation at 10,000 ×g for 15 minutes at 4°C to remove cellular biomass. Biosurfactant compounds were recovered from the cell-free supernatant through a sequential purification process. Initial concentration was achieved by acid-induced precipitation at pH 2.0, followed by liquid-liquid extraction employing a chloroform-methanol mixture (2:1 ratio). The organic phase containing the target compounds was concentrated to dryness using rotary evaporation maintained at 40-45°C, yielding crude biosurfactant preparations [10].

2.4 Structural Characterization

Structural characterization of the biosurfactants involved complementary analytical techniques. Fourier-transform infrared spectroscopy was conducted employing a Shimadzu IRTracer-100 spectrophotometer. Sample preparation involved homogenization with potassium bromide followed by compression into

translucent pellets. Spectral acquisition covered the wavelength range of 4000-400 cm^{-1} to identify characteristic functional groups [11]. Complementary analysis utilized gas chromatography-mass spectrometry with an Agilent 7890B chromatograph interfaced with a 5977A mass selective detector. Compound separation was accomplished using an HP-5MS capillary column, with the oven temperature progressively increased from 60°C to 300°C at a constant rate of 10°C per minute [12].

2.5 Biosurfactant Activity Assays

The critical micelle concentration was assessed through surface tension measurements of sequentially diluted biosurfactant solutions using a calibrated tensiometer. Emulsification potential was evaluated by combining 2 mL of biosurfactant solution with an equal volume of kerosene, followed by vigorous mixing and subsequent quantification of the stable emulsion layer after 24-hour incubation [13]. Complementary qualitative assessments of surface activity included standardized oil displacement and drop collapse methodologies, performed in accordance with established analytical procedures [14].

3. Results and Discussion

3.1 Molecular Identification and Phylogenetic Analysis

Molecular characterization via 16S rRNA gene sequencing established the taxonomic classification of both microbial isolates. Strain S43 exhibited complete genetic alignment with Pseudomonas aeruginosa strain 902 (Accession No. JQ281110.1), whereas strain S54 displayed 98% sequence homology with Pseudomonas putida strain AM-3 (Accession No. MN508028.1). These taxonomic assignments align with documented occurrences of these bacterial species in hydrocarbon-impacted ecosystems [15,16].

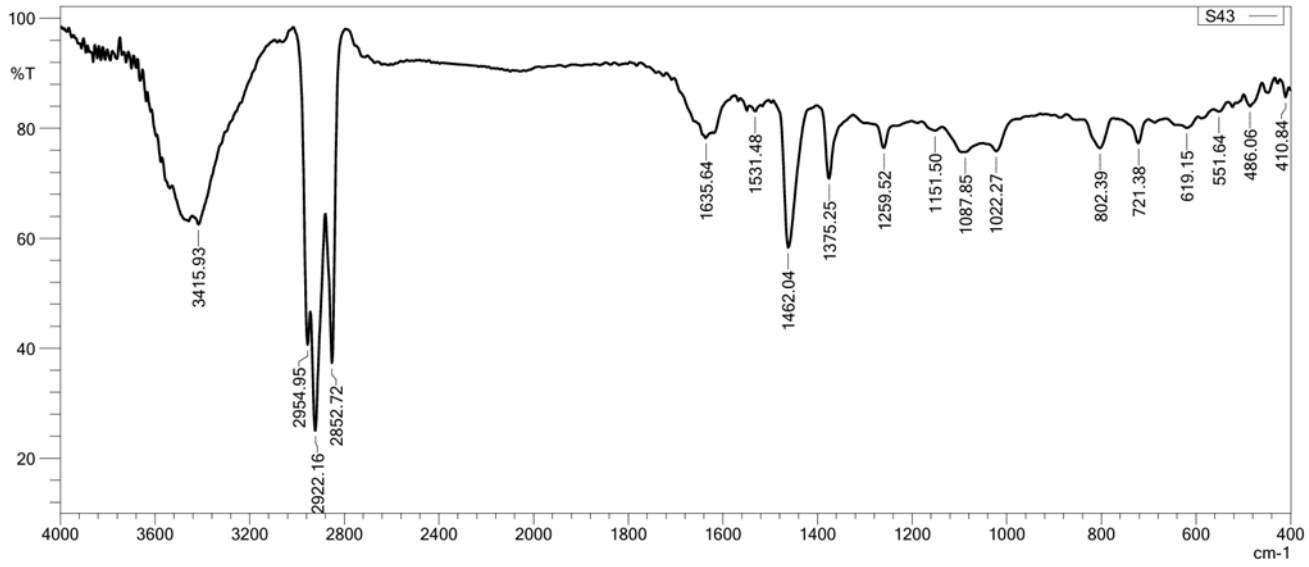
Phylogenetic examination revealed distinct clustering with corresponding reference strains, validating precise taxonomic determination. Although both microorganisms belong to the Pseudomonas genus, they occupy separate phylogenetic branches, indicating divergent evolutionary pathways and ecological specialization. P. aeruginosa demonstrates optimal growth in organic matter-rich habitats and exhibits superior biosurfactant synthesis capability, while P. putida possesses enhanced ecological flexibility and catabolic diversity [17,18].

3.2 FTIR Spectral Analysis

FTIR analysis provided insights into the structural characteristics of the biosurfactants produced by both species

(Figure 1) FTIR for S43 isolate.

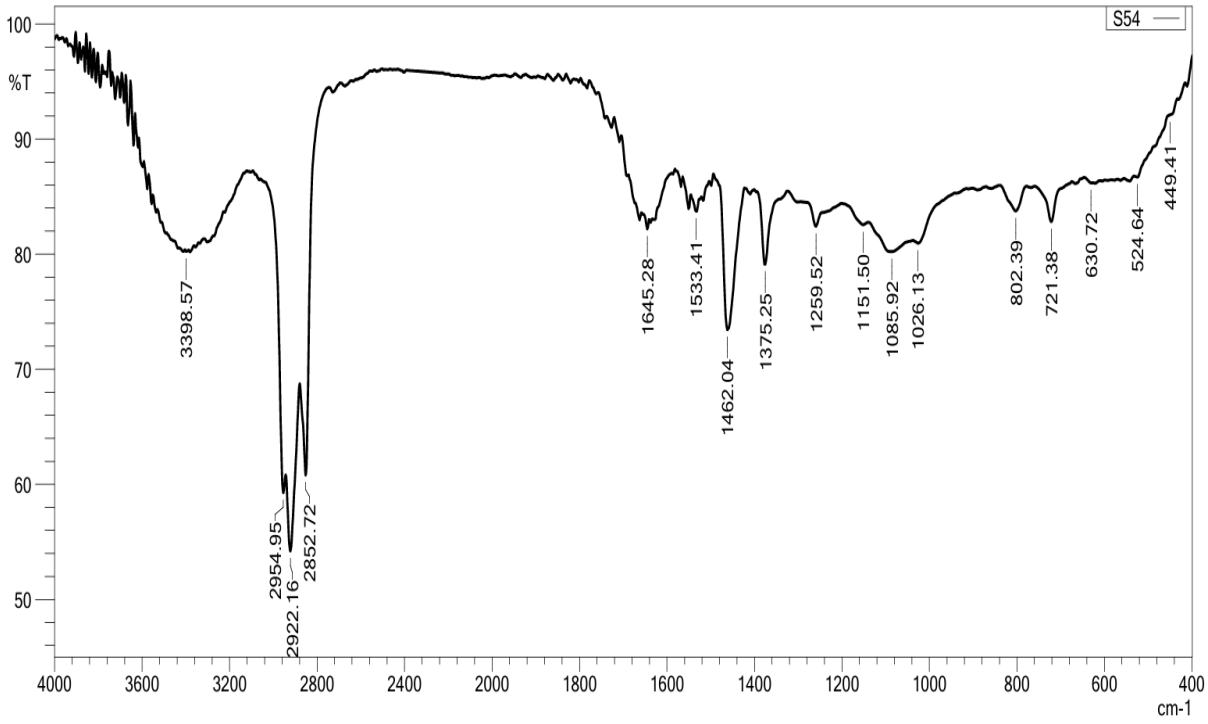
The spectra exhibited consistent absorption patterns with some notable differences:



P. aeruginosa S43 showed characteristic bands at:

- 3415 cm^{-1}: O-H/N-H stretching vibrations
- 2922-2852 cm^{-1}: Aliphatic C-H stretching
- 1720 cm^{-1}: Ester carbonyl groups
- 1635 cm^{-1}: Amide I band
- 1540 cm^{-1}: Amide II band
- 1100 cm^{-1}: C-O-C stretching (glycosidic linkages)

(Figure2) FTIR for S54



P. putida S54 exhibited:

- 3437 cm^{-1}: O-H/N-H stretching vibrations
- 2924-2854 cm^{-1}: Aliphatic C-H stretching
- 1645 cm^{-1}: Strong amide I band
- 1546 cm^{-1}: Amide II band
- 1250-1020 cm^{-1}: C-O stretching vibrations

The presence of strong amide bands in both spectra suggests peptide components, consistent with lipopeptide biosurfactants. However, the more pronounced ester carbonyl band at 1720 cm^{-1} in *P. aeruginosa* S43 indicates a higher proportion of glycolipid-type biosurfactants, particularly rhamnolipids [19]. The detection of glycosidic linkage bands (~1100 cm^{-1}) further supports the glycolipid nature of the *P. aeruginosa* biosurfactant.

3.3 GC-MS Analysis of Biosurfactant Compounds

GC-MS analysis revealed distinct chemical profiles for the biosurfactants from both species (Table 1):

Table 1. Major compounds identified in biosurfactant extracts by GC-MS analysis

Compound	*P. aeruginosa* S43 (%)	*P. putida* S54 (%)
Decanoic acid	12.4	8.9
Dodecanoic acid	14.1	10.2
3-Hydroxydecanoic acid	18.5	-
3-Hydroxydodecanoic acid	9.8	-
Hexadecanoic acid	6.3	9.6
Methyl decanoate	4.2	-
Trimethylsilyl-L-rhamnose	3.1	-
Catechol	-	14.3
Protocatechuic acid	-	2.5
Indole-3-acetic acid	-	0.9
Rhamnose methyl ether	-	0.8

Chromatographic analysis revealed distinct biosynthetic profiles between the two bacterial strains. *P. aeruginosa* S43 primarily generated rhamnolipid compounds, defined by substantial quantities of 3-hydroxy fatty acids and associated rhamnose moieties. This biochemical signature aligns with established rhamnolipid production pathways characteristic of this species [20].

Conversely, *P.putida* S54 demonstrated more complex metabolic activity, synthesizing both conventional biosurfactant elements and notable concentrations of aromatic metabolites. The identification of catechol and protocatechuic acid

implies active engagement in breaking down aromatic hydrocarbons, while the detection of indole-3-acetic acid suggests additional physiological functions that could support plant-assisted remediation approaches [21,22].

3.4 Biosurfactant Activity and Functional Properties

The biosurfactants from both species exhibited significant surface-active properties, though with distinct characteristics (Table 2):

Table 2. Comparative biosurfactant activity parameters

Parameter	*P. aeruginosa* S43	*P. putida* S54
Critical Micelle Concentration (mg/L)	85 ± 9	95 ± 11
Emulsification Index E24 (%)	48.5 ± 4.2	41.7 ± 4.1
Oil-spreading diameter (mm)	65.2 ± 3.1	62.1 ± 3.5
Surface tension reduction (mN/m)	28.5 ± 0.8	32.4 ± 1.2

P. aeruginosa S43 demonstrated superior biosurfactant activity with lower CMC, higher emulsification index, and greater surface tension reduction. These characteristics are consistent with its production of classical rhamnolipids, which are known for their excellent surface-active properties [23]. The lower CMC indicates higher efficiency, requiring less biosurfactant to achieve maximum surface tension reduction.

P. putida S54, while showing slightly reduced surface activity compared to S43, exhibited unique functional advantages. The presence of aromatic degradation intermediates in its biosurfactant extract suggests concurrent hydrocarbon degradation capabilities [24]. This multifunctionality could be particularly valuable in field applications where both emulsification and degradation are required.

3.5 Ecological and Biotechnological Implications

The contrasting biosurfactant properties and functional attributes of the two Pseudomonas species present significant considerations for environmental remediation strategies. *P. aeruginosa* S43 demonstrates exceptional surface-

active performance and efficient rhamnolipid synthesis, making it particularly effective for scenarios demanding rapid hydrocarbon dissolution and increased contaminant accessibility [25]. Nevertheless, its potential pathogenic characteristics may restrict deployment in certain ecological settings.

P. putida S54, despite generating less powerful surfactants, provides multiple benefits including its GRAS certification, wider metabolic diversity, and synthesis of phytostimulatory compounds [26]. The identification of catechol and protocatechuic acid derivatives in its metabolic profile reflects active participation in breaking down aromatic hydrocarbons, positioning it as a valuable candidate for sites polluted with persistent aromatic compounds.

The synergistic potential between these microorganisms indicates promising opportunities for developing combined bioremediation systems [27]. Integrated microbial communities could simultaneously utilize the exceptional surfactant capacity of *P. aeruginosa* and the extensive metabolic range and environmental compatibility of *P. putida*, creating a comprehensive approach to hydrocarbon contamination management.

4. Conclusion

This investigation delineates fundamental contrasts in biosurfactant synthesis and functional performance between the two examined Pseudomonas strains. The S43 strain primarily yielded rhamnolipid compounds demonstrating exceptional interfacial activity, whereas the S54 strain produced metabolically diverse surfactants with concurrent aromatic degradation functions.

Selection of appropriate strains for remediation purposes necessitates careful evaluation of site-specific parameters and cleanup objectives. The S43 variant proves optimal for scenarios demanding intensive hydrocarbon dissolution, while the S54 strain offers advantages in contexts requiring compound mineralization, phytoremediation compatibility, or heightened biological safety.

Subsequent investigations should prioritize the refinement of production methodologies, exploration of multi-strain synergistic effects, and translation of laboratory observations to field implementation. The complementary functional attributes exhibited by these microbial strains establish a foundation for advanced bioremediation approaches targeting petroleum-polluted ecosystems.

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