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Research Article

# Biosurfactant producing abilities of some bacteria isolated from bitumen contaminated soils

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## Abstract

Bio-surfactants produced by bacteria are surface-active compounds required in the degradation of hydrocarbons. They are complex groups of surface-active molecules produced by microorganisms that stick to the specific cell covering or are secreted extracellularly in the growth medium. This study is aimed at determining the bio-surfactant-producing abilities of bacteria that are isolated from bitumen-polluted soil.

Isolation of bacteria from bitumen-polluted soil samples was carried out using standard methods. The bio-surfactant producing ability of the isolated bacteria was investigated by several assays, including drop collapse test, tilting glass slide test, emulsification index, and foaming activity. Characterization of the isolated bacteria was carried out using 16S rRNA, and the extracted genome from each isolate was sequenced, showing the gene annotation of the isolates. Crystal violet biofilm analysis was carried out to determine the biofilm-producing ability of the isolated bacteria. The extracted bio-surfactant was characterized with Fourier Transform Infrared Spectroscopy (FTIR) spectra and Scanning Electron Microscopy (SEM). Whole-genome sequencing analysis was done on two best Bio-surfactant-producing bacteria.

Characterization of the bacteria isolates by 16S rRNA showed their homology in the phylogenetic tree in which *Lysinibacillus sphaericus* belong to the clade of *Lysinibacillus* and *Bacillus*. The biofilm analysis revealed that all the isolates were biofilm producers, with one high producer, three moderate producers and one weak producer. The SEM spectra revealed the structure of the product produced, and FTIR confirmed their chemical nature, indicating rhamnolipids.

The bio-surfactant results indicated that two isolates, *Lysinibacillus sphaericus* and *Pseudomonas sp*. were the best Bio-surfactant-producing isolates.

## Introduction

Bio-remediation points to the application of microbes to debase contaminants that pose a human and environmental threat [1]. Bio-remediation methods typically require many diverse microorganisms operating in likeness or progression to achieve the bio-remediation process. In situ and ex situ (extraction and treating in different place) remediation methods are used. The versatility of microorganisms to degrade a wide collection of pollutants makes bio-remediation a technology that can be used in various soil conditions [1].

Both bacteria and fungi produce some high molecular weight bio-surfactant and emulsifier [2]. Bio-surfactants can be classified under specific polysaccharides, including protein, lipoproteins lipopolysaccharides, and many structural types. Bacterial strains pertaining to the genus *Pseudomonas* and *Bacillus* commonly exhibit lipopeptide bio-surfactant. Virtually every class of microorganism produce bio-surfactants [2]. Synthetic surfactants have the latent disadvantage of persisting in the environment long after applying for a remedial measure due to the xenobiotic characteristics. Also, some of the synthetic surfactants are comparatively more toxic to human health. Bio-surfactants can be regarded as a better



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choice to chemically-synthesized surfactant for environmental clean-up. As microbes typically produce bio-surfactants, researchers have actively been studying them for over a decade [2]. According to Habe and Omori [3], the natural remediation of Poly Aromatic Hydrocarbon-polluted soil should be cost-effective and time-efficient. The biological technique has several benefits which include: complete degeneration of the contaminants, cheaper treatment cost, increased safety, and minor soil disruption.

Till the Critical Micelle Concentration (CMC) is attained, the concentration of surface-active chemicals determines the activity of bio-surfactants [4]. The emulsification of the hydrocarbon in the medium is frequently observed when microorganisms grow on it. This has been attributed to the generation of surface-active chemicals in most situations [4].

The biochemical process of bioremediation has been adopted to reduce environmental pollution and economic loss 2011). It represents the primary mechanism by which petroleum and other hydrocarbon pollutants can be removed from the environment. It is non-invasive and cheaper than other remediation technologies, and a complex process that depends on nature and the amount of hydrocarbon present.

In recent times, *Pseudomonas aeruginosa* is one of the well-known bio-surfactant producing microbes, and they are ubiquitous. Bio-surfactants are used to improve petroleum hydrocarbon degradation efficiency because of advantages such as wide application range, low toxicity, good environmental capability, strong foaming ability and high environmental tolerance [5]. The significance of this research work is to obtain different strains of microorganisms that are capable of bioremediation of biodegraded hydrocarbons.

Bitumen-contaminated soils were selected for this study because it has a larger rate and it needs more action from the bacteria to degrade its component. Bitumen takes about 40 days for total biodegradation depending on the strain of bacteria used. Whereas, petroleum, kerosene and other crude oil fractions take a shorter period. And since the study requires a long time to be carried out effectively, bitumen-contaminated soils were chosen.

New strains of bacteria that have not been recorded before were obtained and recorded in this review.

# Methods

## Preparation of Minimal Salt Medium (MSM)

Minimal salt Medium (MSM) is a medium with the most negligible but necessary nutrients for the growth of bacteria in which carbon source is absent. Bitumen serves as the carbon source during the inoculation of these isolates, which serves as a selective medium for their growth [6]. The composition of MSM was as follows: 0.2g of $MgSO_4$, 0.2g of $CaCl_2$, 1.0g of $KH_2P$, 1.0g of $K_2HP$, 1.0g of $NH_4N$, 0.05g of $FeCl_2$ in 1 liter of distilled water and shaken briskly while 2% agar was added for solidification. This was then placed on a hot plate for proper homogenization before autoclaving at 121°C for 15mins [6].

## Isolation of bitumen degrading bacteria

Ten grams of the bitumen contaminated soil samples were introduced into 100ml of a minimal salt medium, and bitumen was introduced as a carbon source. This was inoculated at 37°C for 48 hours. After 48 hours, 50µl was introduced into the already prepared nutrient agar plates separately and spread, then incubated for 24 hours; distinct colonies were picked and streaked to get a pure colony [7].

## DNA isolation

The isolated bacteria were cultured by streaking on plates containing MSM and was incubated for two days. Visible colonies were produced on the plates. A single colony was inoculated in 1-3 ml of the MSM and allowed to grow in a shaker bath up to the mid-log phase. The cells were collected by centrifugation of 1.5 ml of the culture for 2 min at 8000 rpm in the micro-centrifuge. If the growth of the culture was poor after centrifugation, a second 1.5 ml aliquot of the culture could be centrifuged in the same tube.

The cell was suspended in pellet 500 µl of TE buffer and 30 µl of Sodium Dodecyl Sulfate (SDS) together with five µl of proteinase K. The tube was mixed by inversion and incubated at 37°C for 1 hour to allow cell lysis. 100 µl of 5M NaCl was added and vortexed for few seconds. 80 µl of CTAB was added, mixed and heated for 10 min at 65°C. An equal volume (about 800 µl) of chloroform: isoamyl alcohol was added, vortexed for a few seconds and centrifuged for 5 min at 11000 rpm. The aqueous upper phase was collected in a new tube, and an equal volume of phenol:chloroform: isoamyl alcohol was mixed by vortex and centrifuged for 5 min at 11000 rpm. The upper aqueous phase was recovered in a fresh tube, two µl of RNAs was added and incubated for 30 min at 37°C. An equal volume of isopropanol was added, DNA precipitated for 5 min at room temperature and centrifuged for 5 min at 11000 rpm. The supernatant was discarded, and the pellet was washed with 70% ethanol and centrifuged again for 5 min at 11000 rpm. The pellet was dried under vacuum and solubilized in 10-20 µl of sterile TE buffer solution.

## Molecular identification of bacteria isolates

The genetic identity of bacterial isolates was determined by isolating and amplifying DNA sequence data from 16S rRNA. The 16S rRNA sequence has been established as the primary bacterial barcode (Schoch, *et al*. 2012).

## Phylogenetic characterization

The amplified products were sequenced in line with the Big Dye Terminator sequencing procedure in an Abl 377 programmed DNA Sequencer (Applied Biosystems, USA) 16S rRNA sequence reads obtained after sequencing were assembled into contig with Geneious alignment tool. Each contig sequence was a blast on NCBI (National Center for Biotechnology Information) database for identification; some other sequences were searched and downloaded based on each query sample genus name and query covered.



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Alignment of all sequences, including the query sequence, was conducted with the Muscle Alignment tool. A phylogenetic tree was constructed using Neighbor-Joining and the maximum likelihood base on genetic distance model with a bootstrap value of 1000 and 100 numbers of replicates to indicate the revolutionary process analyzed over time. Geneious tree builder version 9.0.5 was used to edit and to determine the evolutionary relatedness and diversities.

## Fourier transform infrared spectroscopy

To understand the overall chemical nature of the extracted bio-surfactant, Fourier transforms infrared (FTIR) spectroscopy was employed. The technique helps to explore the functional groups and the chemical bonds present in the crude extract [8]. The analysis was done using Shimadzu FTIR Spectrophotometer (Model 8400S). Samples were prepared by homogeneous dispersal of 1 mg of the bio-surfactant sample in potassium bromide pellets (Merck, USA). IR absorption spectra were obtained using a built-in plotter. IR spectra were collected over the range of 450–4500 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. The spectral data were the average of 50 scans over the entire range covered by the instrument. The spectrum was studied to interpret the chemical nature of the bio-surfactant fraction [8].

## Screening of bio-surfactant producing isolates

Bacteria were cultivated aerobically in 500 ml Erlenmeyer flask with 100 ml of mineral salt medium containing ($gl^{-1}$) 1.0 $K_2HPO_4$, 0.2 $MgSO_4.7H_2O$, 0.05 $FeSO_4.7H_2O$, 0.1 $CaCl_2.2H_2O$, 0.001 $Na_2MoO_4.2H_2O$, 30 Sodium Chloride and crude oil (1.0%, w/v). Flasks holding purified mineral salt medium were introduced with a loopful bacterial culture grown in crude oil containing nutrient agar plates [9]. The culture flasks were shaken for seven days at 200 rpm and 30°C. After seven days of incubation, culture broth from each flask was centrifuged at 6000 rpm and 4°C for 15 minutes and the supernatant was filtered through 0.45µm pore size filter paper (Millipore). This cell-free culture broth was used for drop collapse assay, oil spreading assay, emulsification assay and surface tension measurement [10]. All the screening experiments were carried out in triplicates (except otherwise stated).

## Drop collapse test

Two liters of crude oil were poured into the wells of a polystyrene 96-well microplate cap and allowed to dry for 24 hours at 22°C. The center of the oil-coated well was filled with filtered cell-free supernatant (5 L). When the oil drop was flat after 1–2 minutes, the results were recorded and considered positive for bio-surfactant production. Those who produced rounded drops received a negative score, indicating a lack of bio-surfactant production [11].

## Emulsification index

In a 30 mL screw-capped test tube, 5 mL of 50 mM Tris buffer was added to 1 mL of cell-free supernatant (pH 8.0). The ability of crude oil to emulsify was investigated [12]. Both layers received 5 mg of crude oil, which was vortexed for 1 minute before the emulsion mixture was allowed to resolve for 20 minutes. The emulsified mixture's optical density was measured at 610 nm [12]. The negative control was buffered, while the positive control was crude oil with Triton X-100.

Emulsification index= (emulsion layer height/total height)/100

$$Emulsification\ Index = \frac{Emulsification\ layer\ height}{Totalheight}$$

## Tilting glass slide test

In the tilting glass slide test which was developed by Persson and Molin [13], a single colony is picked up from the Bushnell Haas agar plate and transferred on the surface of a sterile glass slide near one of the edges. It is then mixed with a droplet of 1% saline. The slide is gradually tilted to the other side and was examined for the flow of a water droplet over its surface. Bio-surfactant production is implied if water flows over the surface.

## Phenol sulfuric acid assay

The presence of carbohydrate groups in the bio-surfactant molecule was assayed using this standard method. A volume of 0.5 ml of culture supernatant was mixed with 0.5 ml of 5% phenol solution and 2.5 ml of sulfuric acid and incubated for 15 min before measuring absorbance at 490 nm [14].

## Surface tension measurement

Bacterial strains that exhibited positive results for bio-surfactants production were then evaluated for surface tension reduction and stable emulsion formation. Strains were grown in MSM broth with crude oil 2% (v/v) and incubated at 200 rpm for seven days. For surface tension measurements, 5ml of broth supernatant was transferred to a glass tube submerged in a water bath at constant temperature (28°C). Surface tension was calculated by measuring the height reached by the liquid after freely ascending through a capillary tube. As a control, broth without inoculation was used. The experiments were carried out with three independent replicates [10].

## Foaming activity

Foaming ability was determined by growing the cultures in 50 ml nutrient broth in a 250 ml Erlenmeyer flask. It was incubated at 30°C for 96 hours on a shaker incubator [15]. The 10 ml of culture was shaken vigorously for 2 min in a graduated cylinder, and the equation detected foaming activity.

$$Foaming = \frac{Height\ of\ \ foam}{Total\ height} x\,100$$

## Bacterial adhesion to hydrocarbon assay

Cell hydrophobicity was measured by bacterial adherence to hydrocarbons according to a method described by Rosenberg, *et al*. 1980. The cell pellets were washed twice and suspended in a buffer salt solution (16.9 g/l $K_2HPO_4$ and 7.3g/l $KH_2PO_4$) and diluted using the same buffer solution to an optical density



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(OD) of ~ 0.5 at 610 nm [10]. To the cell suspension (2 ml) in test tubes (10 ml volume with 10 x 100 mm dimension), 100 µl of crude oil was added and vortex-shaken for 3 min. After shaking, crude oil and aqueous phases were allowed to separate for 1 hour. OD of the aqueous phase was then measured at 610 nm in a spectrophotometer [10]. From the OD values, the percentage of cells attached to crude oil was calculated using the following formula.

$$\%of\ bacterial\ cell\ adherence = (1 - (\frac{OD_{shaken\ with\ oil}}{OD_{original}})) X\ 100$$

Where: $OD_{shaken\ with\ oil}$ is the OD of the mixture containing cells and crude oil

$OD_{original}$ is the OD of the cell suspension in the buffer solution (before mixing with crude oil).

## Results

### Contaminated soil sample

The 16S rRNA (ribosomal RNA) gene sequencing of the isolates were identified as *Brucella intermedia* (6A1), *Pseudomonas aeruginosa* (8AA1), *Kocuria indica* (LB1P4), *Lysinibacillus sphaericus* (LD15A), *Pseudomonas sp*. (LC1PC5) with NCBI blast. The neighbor-joining analysis showed that isolate LD15A was close to the clade of *Lysinibacillus and Bacillus*; 8AA1 belonged to the clade of *Ochrabacterium*, LC1PC5 was close to the *Pseudomonas aeruginosa*, while LB1P4 belonged to the clade of *Kocuria* (Figure 1). The accession numbers of the five isolates as presented in Table 1 depict 6A1 as *Brucella intermedia* (MW677449), 8AA1 as *Pseudomonas aeruginosa* (MW677446), LB1P4 as *Kocuria indica* (MW677447), LC1PC5 as *Pseudomonas sp*. (MW677450), and LD15A as *Lysinibacillus sphaericus* (MW677448). Figure 2 shows the annotation of LC1PC5 (*Pseudomonas sp*.) RBS Kozak, T7 trans en RBS and Casp-3, Thromb Tag and others were found in the DNA. They are a nucleic acid motif that functions as the protein translation initiator in most eukaryotic mRNA transcripts. Regarded as the optimum sequence for initiating translation in eukaryotes, the sequence is an integral aspect of protein regulation and overall cellular health and has implications in human disease.

Gene annotation was carried out to help in the identification

**Table 1:** Molecular Characterization (16S rRNA) of the bacterial Isolates.

| Isolate code | Isolate Identity | Ascension Number |
|---|---|---|
| 6A1 | *Brucella intermedia* | MW677449 |
| 8AA1 | *Pseudomonas aeruginosa* | MW677446 |
| LB1P4 | *Kocuria indica* | MW677447 |
| LC1PC5 | *Pseudomonas* sp. | MW677450 |
| LD15A | *Lysinibacillus sphaericus* | MW677448 |

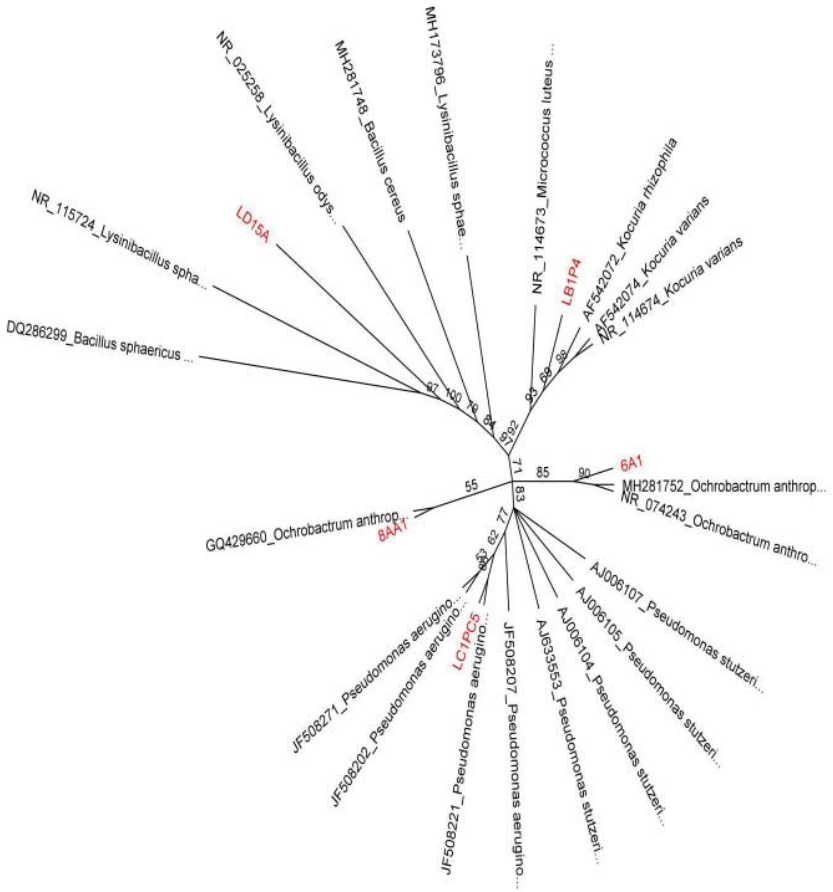


**Figure 1:** Phylogenetic Relationship of Isolated Bacteria from Bitumen-Contaminated Soil Sample.



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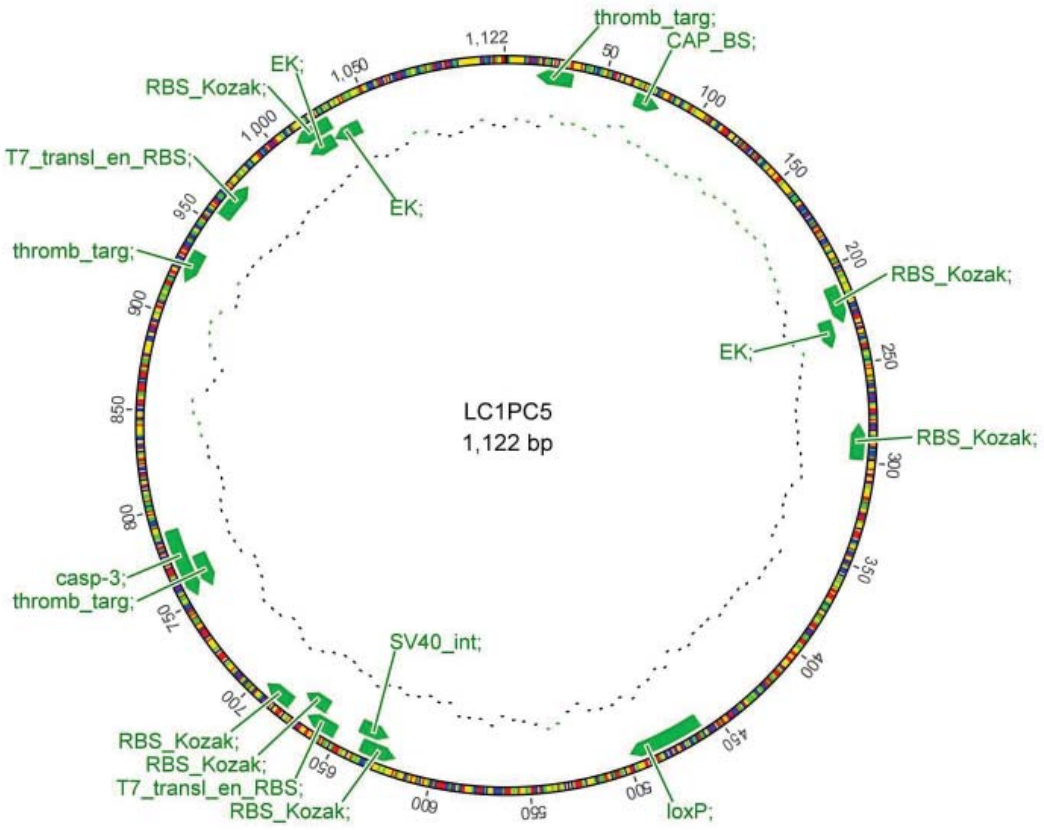


**Figure 2:** Gene Annotation of *Pseudomonas* sp.

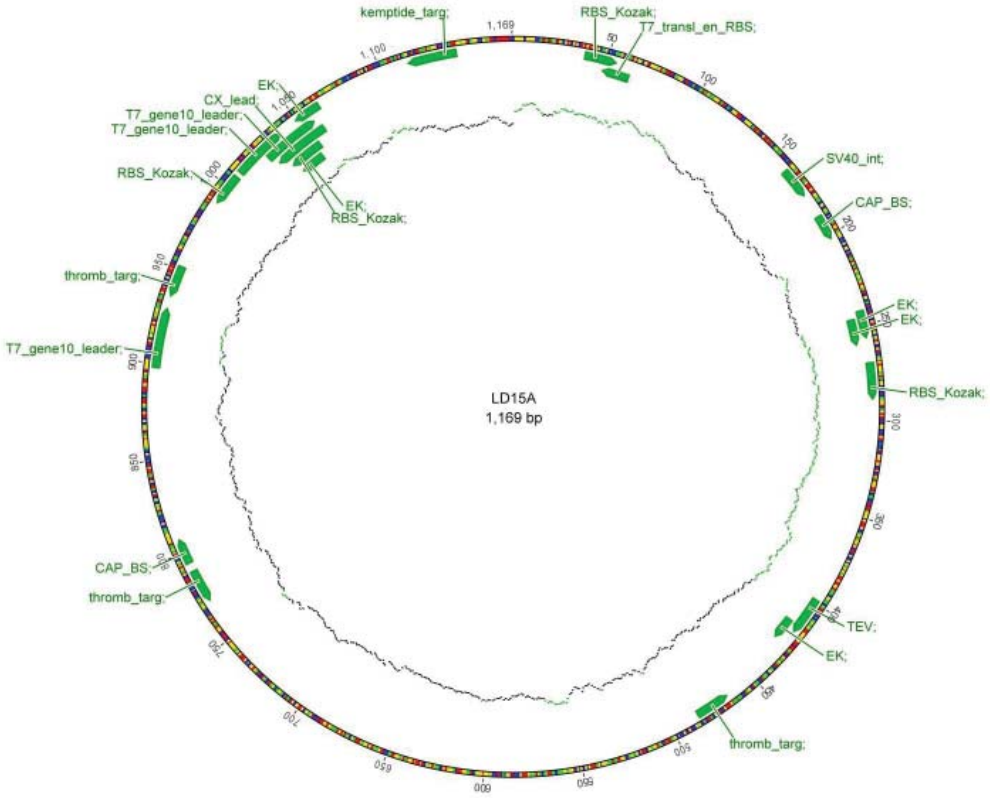


**Figure 3:** Gene Annotation of *Lysinibacillus sphaericus*.



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of gene location and coding regions. It also helps to give an insight on what these genes do in the body by establishing structural aspects and relating them to functions of different proteins.

Figure 3 shows the annotation of LD15A (*Lysinibacillus sphaericus*), RBS Kozak, T7- transl-en-RBS and Casp-3, Thromb Tag and others were found in the DNA. They are a nucleic acid motif that functions as the protein translation initiator in most eukaryotic mRNA transcripts. Regarding the optimum sequence for initiating translation in eukaryotes, the sequence is an integral aspect of protein regulation and overall cellular health.

*Brucella intermedia* was positive for the drop collapse test, negative for the tilting glass slide test and positive for the sulphuric acid test. *Pseudomonas aeruginosa* was negative for the drop collapse test and tilting glass slide test but positive for the sulphuric acid test. *Kocuria indica* was negative for the drop collapse test but positive for both the tilting glass slide test and sulphuric acid assay. *Lysinibacillus sphaericus* is positive for the three tests showing a better ability than other isolates. *Pseudomonas sp*. was negative only for the tilting glass slide test (Table 2).

Table 3 showed that *Pseudomonas aeruginosa* had the highest surface tension while *Lysinibacillus sphaericus* had a low foaming activity with a moderate surface tension of 42cm.

**Table 2:** Drop Collapse, Tilting Glass Slide and Sulphuric Acid Tests of Isolates.

| Isolates | Drop collapse | Tilting glass slide | Sulfuric Acid |
|---|---|---|---|
| *Brucella intermedia* | Positive | Negative | Positive |
| *Pseudomonas aeruginosa* | Negative | Negative | Positive |
| *Kocuria indica* | Negative | Positive | Positive |
| *Lysinbacillus sphaericus* | Positive | Positive | Positive |
| *Pseudomonas sp.* | Positive | Negative | Positive |
| *Consortium* | Positive | Positive | Positive |

**Table 3:** Test on Foaming Activity and Surface Tension of Isolates.

| Isolates | Foaming activity (cm) | Surface tension (cm) |
|---|---|---|
| *Brucella intermedia* | 1.63±2.05 ab | 40.00±1.00 a |
| *Pseudomonas aeruginosa* | 1.20±0.10 ab | 47.00±1.00 b |
| *Kocuria indica* | 1.00±0.10 ab | 45.00±1.00b |
| *Lysinibacillus sphaericus* | 0.33±0.06 a | 42.00±2.00 a |
| *Pseudomonas sp.* | 1.60±0.10 ab | 40.00±1.00 a |
| *Consortium* | 2.50±0.10 b | 40.67±1.53 a |

**Table 4:** Biofilm Forming Ability of Isolates by Crystal Violet Biofilm Test.

| Isolates | Crystal violet Test |
|---|---|
| *Kocuria indica* | 0.85±0.003e |
| *Pseudomonas aeruginosa* | 0.22±0.002b |
| *Lysinibacillus sphaericus* | 0.28±0.001c |
| *Pseudomonas sp.* | 0.11±0.001a |
| *Brucella intermedia* | 0.29±0.002d |
| *Consortium* | 0.10±0.003e |

**Table 5:** Classification of Isolates Based on their Biofilm Forming Ability.

| Biofilm Production Ability | No of Isolates (%) | Biofilm Yield Range |
|---|---|---|
| Strong | 1 (20) | >0.412 |
| Moderate | 3 (60) | 0.206-0.412 |
| Weak | 1 (20) | 0.103-0.206 |

*Kocuria indica* showed a surface tension of 45cm with foaming activity of 1cm. *Brucella intermedia* and *Pseudomonas sp*. showed the lowest surface tension.

The Biofilm-producing ability of the isolates, as shown in Table 4, revealed a significant difference in their biofilm-producing ability. *Kocuria indica* produced the highest biofilm (0.85), while *Pseudomonas sp*. produced the least (0.11).

Table 5 showed the classification of bacteria isolates based on their biofilm-forming ability. *Kocuria indica* was a strong biofilm production ability having a yield greater than 4x $OD_{cut}$ (0.412). Three isolates were moderate biofilm producers, and these include *Pseudomonas aeruginosa*, *Lysinibacillus sphaericus* and *Brucella intermedia*. Only *Pseudomonas sp*. was a weak producer.

The emulsification index of the isolates, as shown in Figure 4, revealed a significant difference in their ability to produce an emulsion. For *Brucella intermedia*, *Pseudomonas aeruginosa*, *Kocuria indica*, *Lysinibacillus sphaericus*, *Pseudomonas sp*., and *Consortium*, the Emulsification Index obtained were 43.20%, 35.00%, 31.30%, 44.12%, 41.86% and 29.54%, respectively.

Figure 5 showed the percentage of cells bound to the hydrocarbon phase. For *Brucella intermedia*, the percentage of cells bound to the hydrocarbon phase was high on the 5th (62.2%) day, but its percentage dropped a little on day 10 (61.5%) and by 0.1% on day 15 (61.6%). *Pseudomonas aeruginosa* had a low percentage of cells (8.86%) bound to the hydrocarbon phase on the 5th day, equivalent to the increase in turbidity of the aqueous phase. On day 10, it increased to 9.8% and dropped to 9.3% on day 15, showing that the isolates *Pseudomonas aeruginosa* had a small number of bio-surfactants producing microbes. *Kocuria indica* had the highest percentage at day 5 (49.7%), then it dropped at day 10 to 49.2% and increased a little at day 15 to 49.6%. *Lysinibacillus sphaericus* had its highest percentage on days 5 and 15, which is (64.8%). For *Pseudomonas sp*., the percentage of cell-bound to hydrocarbon assay increases gradually from day 5 to day 15 (70.1 to 70.4 to 70.6%). The *Consortium* had a constant percentage (66%) from day 5 to day 15.

The C-H bending band of *Brucella intermedia* was observed in the region 1435$cm^{-1}$ with a peak height of 6.6458cm. The product also contains residual water vapor, observed in the region 1839$cm^{-1}$ with a peak height of 7.6072cm. the $NH_2$ amino acid group was observed at two regions; 3222 $cm^{-1}$ and 3276 $cm^{-1}$ at the peak heights of 1.2827cm and 1.1747cm, respectively (Figure 6)

The H-O-H stretching vibration of a cluster of water molecules of crystallization of *Pseudomonas aeruginosa* was observed in the region 1636$cm^{-1}$. Residual water vapor was also



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a constituent of the product, which was proved at 1804cm$^{-1}$ with a peak height of 5.9052cm. C-O-C polysaccharides were observed at peak 1127 cm$^{-1}$ with a height of 8.7747cm. Benzene ring ortho-substituted and meta-substituted with condensed ring system were observed at the peak of 736cm$^{-1}$ and a peak height of 17.7787cm (Figure 7).

The C=O Ester Fatty acid group of the consortium was proved from the band at 1724cm$^{-1}$. There is the presence of a double conjugate bond (C=C) with a peak at 2010cm$^{-1}$ and a peak height of 12.3985cm. It contains ash in coal at the peak of 993cm$^{-1}$ and a peak height of 9.9490cm. C-H of the aromatic ring was proved at 2990 cm$^{-1}$ with a peak height of 8.9966cm (Figure 8).

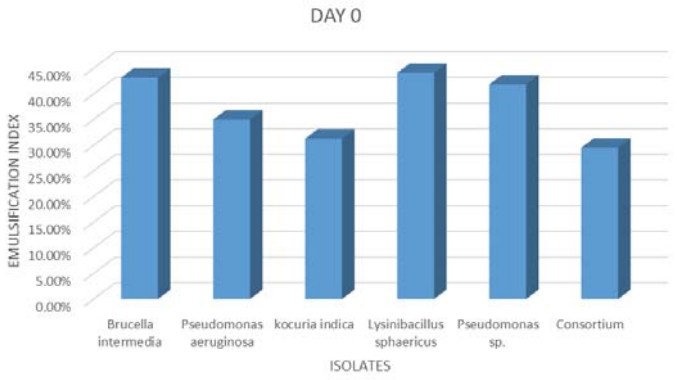


**Figure 4:** Emulsification Index of Isolates.

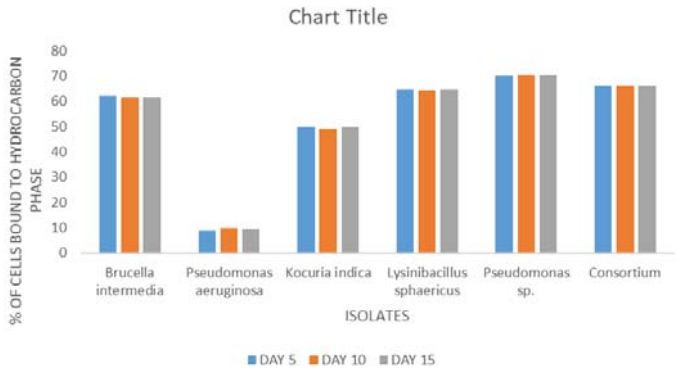


**Figure 5:** Bacterial Adhesion to Hydrocarbon Assay.

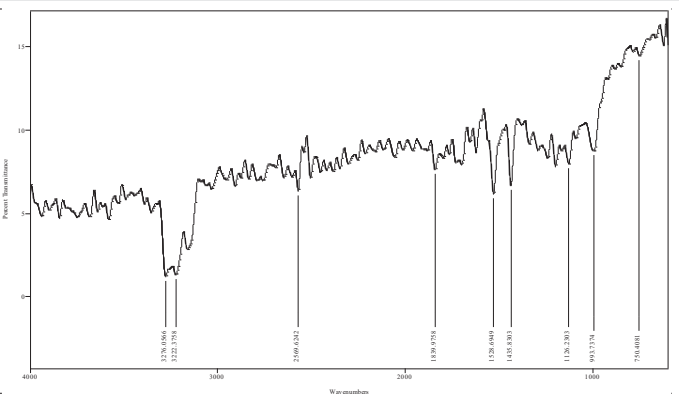

**Figure 6:** FTIR for *Brucella intermedia*-produced Bio-surfactant.

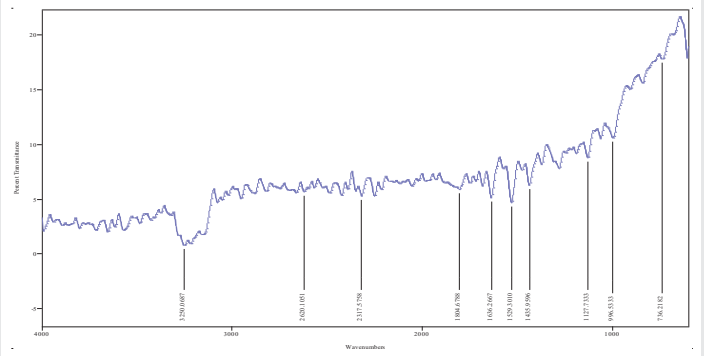

**Figure 7:** FTIR for *Pseudomonas aeruginosa*-produced Bio-surfactants.

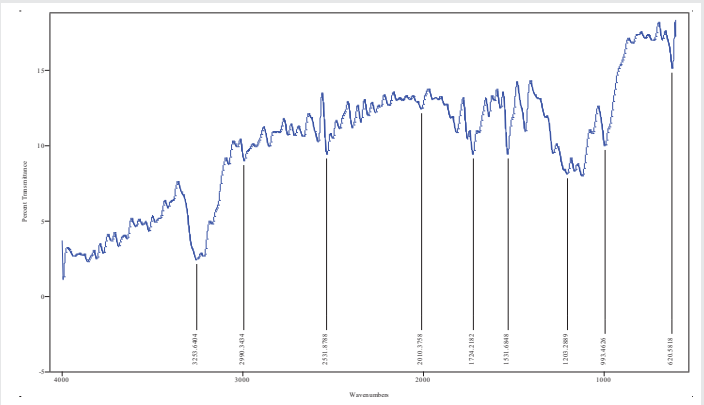

**Figure 8:** FTIR for *Consortium*-produced Bio-surfactant.

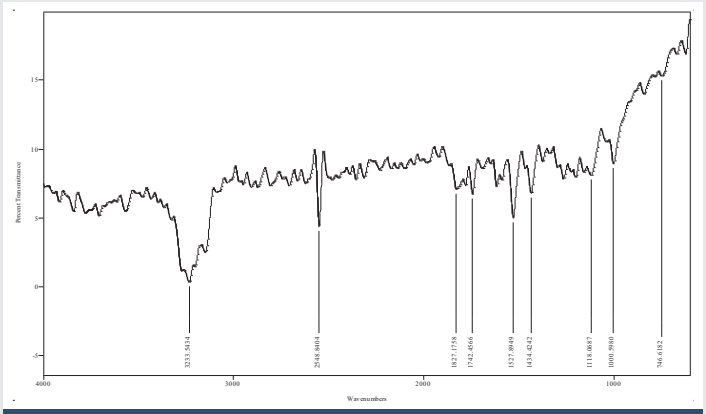

**Figure 9:** FTIR for *Kocuria indica*-produced Bio-surfactant.

The C=O Ester Fatty acid group of *Kocuria indica* was proved from the band at 1742 cm$^{-1}$ with a peak height of 6.7274cm. The C-H bending aliphatic band was also observed at peak 1434 cm$^{-1}$ and a peak height of 6.7639cm. Residual water vapor was also a constituent of the product, observed at the region 1827cm$^{-1}$ with a peak height of 7.0581cm (Figure 9).

The stretching band of *Pseudomonas sp.* was observed at 1432cm$^{-1}$ with a peak height of 11.7284cm. Benzene ring ortho-substituted and meta-substituted with condensed ring system were observed at the peak of 732cm$^{-1}$ and a peak height of 19.3488cm. The ash in coal was observed at the region 992cm$^{-1}$ with a peak height of 13.1187cm (Figure 10).

The stretching band of *Lysinibacillus sphaericus* was proved



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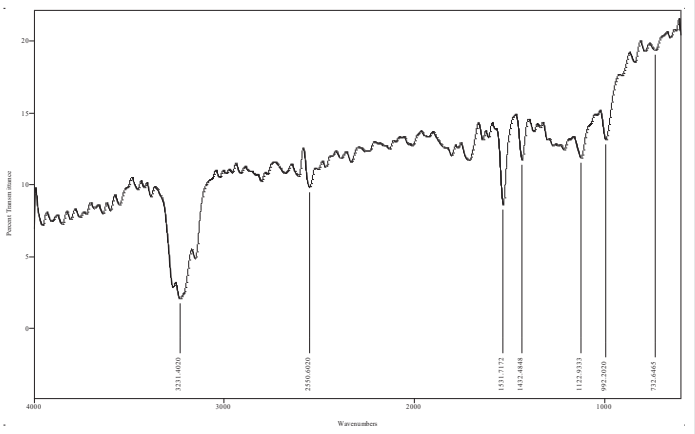


**Figure 10:** FTIR for *Pseudomonas sp.*-produced Bio-surfactant.

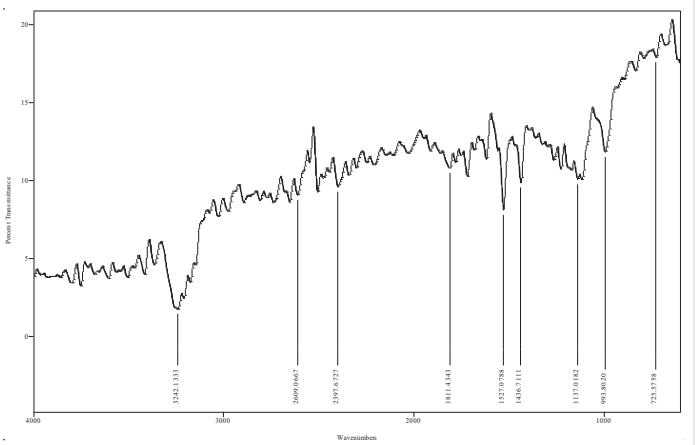


**Figure 11:** FTIR of *Lysinibacillus sphaericus.*

at 1436cm$^{-1}$ and a peak height of 9.8447cm. Residual water vapor was also a constituent of the product, observed at the region 1811cm$^{-1}$ with a peak height of 10.8085cm. Benzene ring ortho-substituted and meta-substituted with condensed ring system were observed at the peak of 725cm$^{-1}$ and a peak height of 17.9243cm (Figure 11).

## Discussion

A total of 5 different bacterial were isolated from the bitumen-contaminated soil samples. The 5 bacterial isolates were identified as *Brucella intermedia*, *Pseudomonas aeruginosa*, *Kocuria indica*, *Lysinibacillus sphaericus* and *Pseudomonas sp.* This result is different from what Amao, *et al.* [16] reported. Three of the bacterial isolates obtained in his work belong to the genus *Bacillus*, one was *Lactobacillus planetarium*, while four others were *Klebsiella Pneumoniae*. This may be due to the source of samples used in the two studies.

Dans and Chandran (2011) described the drop collapse test according to which the degree of collapse of the culture supernatant describes the surfactant concentration. Of the six samples, two samples show no complete collapse, while for the four other samples, the drops turned flat. The tilted glass slide test, developed by Persson and Molin [13], was positive for three isolates.

Isolate *Lysinibacillus sphaericus* followed by *Brucella intermedia* exhibited the highest emulsification capacity; they gave 44.12±0.02 and 43.20±0.02 of E24%, respectively in this study which agrees with the work of Sidkey, *et al.* (2016). Dussan and Numpaque (2012) had reported that *Pseudomonas sp.* is a useful candidate for bio-surfactant production. Sarubbo, *et al.* (2016) concluded that the emulsification index (E24) provides a rapid and reliable measure of the quantity of Bio-surfactant. *Lysinibacillus sphaericus* had a low foaming activity which is less than 0.5, which correlates with the work of El-sheshtawy (2011). The percentage of cells bound to the hydrocarbon phase showed a characteristic feature of Bio-surfactant-producing microbes. *Pseudomonas sp.* showed the highest percentage, while *Pseudomonas aeruginosa* showed the lowest percentage.

*Brucella intermedia, Lysinibacillus sphaericus and Pseudomonas sp.* are the best known bacterial groups for biosurfactant-producing genera (Suwansukho, *et al.* 2008), and they were also found in our screening. The majority of the isolated strains belonged to the genus *Bacillus, Brucella* and *Pseudomonas*. These are frequently isolated from hydrocarbon-contaminated environments. Many strains belonging to these genera have been demonstrated to be efficient hydrocarbon degraders and bio-surfactant-producing bacteria [17]. The isolates *Lysinibacillus sphaericus, Pseudomonas sp., Brucella intermedia, Kocuria indica* and *Pseudomonas aeruginosa* exhibited high homology with *Lysinibacillus and Bacillus*, *Ochrabacterium*, *Ochrabacterium*, *Kocuria* and *Pseudomonas aeruginosa*, respectively, which correlate with the work of Saisa-ard, *et al.* (2014).

The Fourier Transform Infrared Spectroscopy (FTIR) spectra of the samples range from 500-4000cm$^{-1}$. The peak of LD15A (*Lysinibacillus sphaericus*) in the region of 1137cm$^{-1}$ indicates C-O-C stretching in the rhamnose. A similar result was reported by Thenmozhi, *et al.* (2011). Rhamnolipids produced by *Lysinibacillus sphaericus* were the most studied bio-surfactants due to their potential applications in a wide variety of industries and high levels of production [18-25]. Rhamnose, in which one or two molecules of rhamnose are linked to one or two molecules of β-hydroxy-decanoic acid, is the best-studied glycolipids. Production of rhamnose-containing glycolipids was first described in *Pseudomonas aeruginosa*. The findings of this study were in accordance with Rahman, *et al.* (2007), who reported bio-surfactant production by *P.aeruginosa* DS10-129 characterized by FTIR technique belonged to rhamnolipid type. Rhamnolipids produced from *Lysinibacillus sphaericus* were characterized, and its ability for dissolution of hydrophobic pesticides was evaluated. It produced 1.6g/L of an anionic bio-surfactant that reduced surface tension. The Bio-surfactant was found stable over a wide range of pH, temperature, salt concentration and was identified as rhamnolipids reported by Vivek, *et al.* (2019).

## References

1. Sylvia DM, Fuhrmann JF, Hartel PG, Zuberer DA (2005) Principles and Applications of Soil Microbiology. New Jersey, Pearson Education Inc. **Link:** https://bit.ly/3AS8TAY

2. Thivaharan V, Vytla M (2013) Production of a Lipopeptide Biosurfactant by a Novel Bacillus sp. and Its Applicability to Enhanced Oil Recovery. **Link:** https://bit.ly/2YXmlXJ



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3. Habe H, Omori T (2013) Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. Biosci Biotechnol Biochem 67: 225–243. Link: https://bit.ly/3ARcq2w

4. Paul Beulah BF, Reena A, Uma Maheswari M (2018) Screening and extraction of biosurfactant producing bacteria from oil contaminated soils. Int J Adv Res 6: 1873-1878. Link: https://bit.ly/3p92JdP

5. Xue J, Wu Y, Shi K, Xiao X, Gao Y, et al. (2019) Study on the degradation performance and kinetics of immobilized cells in straw-alginate beads in the marine environment. Bioresour Technol 280: 88-94. Link: https://bit.ly/3aTnaTr

6. Tahhan RA, Ammari TG, Goussous SJ, Al-Shdaifat HI (2011) Enhancing the biodegradation of total petroleum hydrocarbons in oily sludge by a modified bioaugmentation strategy. International Biodeterioration & Biodegradation 65: 130-134. Link: https://bit.ly/3aOXirT

7. Olowomofe TO, Oluyege JO, Aderiye BI, Oluwole OA (2019) Degradation of poly aromatic fractions of crude oil and detection of catabolic genes in hydrocarbon-degrading bacteria isolated from Agbabu bitumen sediments in Ondo State. AIMS Microbiol 5: 308-323. Link: https://bit.ly/30waMa9

8. Varadavenkatesan T, Ramachandra MV (2013) Production of a Lipopeptide Biosurfactant by a Novel Bacillus sp. and Its Applicability to Enhanced Oil Recovery. International Scholarly Research Notices 2013: 621519. Link: https://bit.ly/3BTArHB

9. Nayarisseri A, Sing P, Singh SK (2018) Screening, isolation and characterization of biosurfactant producing Bacillus subtilis strain ANSKLAB03. Link: https://bit.ly/3ASHRK3

10. Nayarisseri A, Singh P, Singh SK (2018) Screening, isolation and characterization of biosurfactant producing Bacillus subtilis strain ANSKLAB03. Bioinformation 14: 304-314. Link: https://bit.ly/3aMTUhj

11. Mamta R, Joel TW, Suha J (2020) Isolation and Characterization of Biosurfactant-Producing Bacteria From Oil Well Batteries With Antimicrobial Activities Against Food-Borne and Plant Pathogens. Frontiers in Microbiology 64. Link: https://bit.ly/2YWKWvo

12. Muthezhilan R, Ragul R, Pushpam AC, Hussain AJ (2014) Production and purification of Biosurfactant from marine yeast isolated from kelambakkam salterns. Biosci Biotechnol Res Asia 11: 59–67. Link: https://bit.ly/3aKKbrF

13. Persson A, Molin G (2010) Capacity for biosurfactant production of environmental Pseudomonas and Vibrionaceae growing on carbohydrates. Applied Microbiology and Biotechnology 26: 439–442. Link: https://bit.ly/3jblTfl

14. Saikia RR, Deka S, Deka M, et al. (2012) Isolation of biosurfactant-producing Pseudomonas aeruginosa RS29 from oil-contaminated soil and evaluation of different nitrogen sources in biosurfactant production. Ann Microbiol 62: 753-763. Link: https://bit.ly/3aOkiaI

15. Seoud MA, Maachi R, Amranec A, Boudergua S, Nabi A (2008) Evaluation of different carbon and nitrogen sources in Biosurfactant production by Pseudomonas fluorescens. Desalination 223: 143-151. Link: https://bit.ly/3BS0ZJr

16. Amao JA, Omojasola PF, Barooah M (2019) Isolation and characterization of some exopolysaccharide producing bacteria from cassava peels heaps. Scientific African 4: e00093. Link: https://bit.ly/3pcAjj9

17. Ruggeri C, Franzetti A, Bestetti G, Caredda P, La Colla P, et al. (2009) Isolation and characterization of surface-active compound producing bacteria from hydrocarbon-contaminated environments. International Biodeterioration and Biodegradation 63: 936-942. Link: https://bit.ly/3AMgNvL

18. Toribo J, Escalante AE, Soberon-Chavez G (2010) Rhamnolipids: Production in bacteria other than Pseudomonas aeruginosa. Euro J Lipid Sci Technol 112: 1082-1087. Link: https://bit.ly/3aQf8eh

19. Tabatabee A, Mazaheri MA, Noohi AA, Sajadian VA (2005) Isolation of Biosulfactant-producing bacteria from oil reservoirs. Iran J Environ Health Sci Eng 2: 6-12. Link: https://bit.ly/3n11WsF

20. Tremblay J, Yergeau E, Fortin N, Cobanli S, Elias M, et al. (2017) Chemical dispersants enhance the activity of oil-and-gas condensate-degrading marine bacteria. ISME J 11: 2793–2808. Link: https://go.nature.com/3FWWAaw

21. Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73: 1163-1172. Link: https://bit.ly/3DNL4MM

22. Varjani SJ, Gnansounou E (2017) Microbial dynamics in petroleum oilfields and their relationship with physiological properties of petroleum oil reservoirs. Bioresour Technol 245: 1258–1265. Link: https://bit.ly/3aKJK0v

23. Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, et al. (2014) Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med 370: 2408-2417. Link: https://bit.ly/3p8BfF7

24. Yang Y, Wang J, Liao J, Xie S, Huang Y (2015) Abundance and diversity of soil petroleum hydrocarbon-degrading microbial communities in oil exploring areas. Appl Microbiol Biotechnol 99: 1935–1946. Link: https://bit.ly/3pdqXDK

25. Yakimov MM, Timmis KN, Golyshin PN (2007) Obligate oil-degrading marine bacteria. Curr Opin Biotechnol 18: 257–266. Link: https://bit.ly/3AQLtMz






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