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 alternative to synthetic surfactants.
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 isoopropanol 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 evolutionary process analyzed over time. Gennial 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 K₂HPO₄, 0.2 MgSO₄.7H₂O, 0.05 FeSO₄.7H₂O, 0.1 CaCl₂.2H₂O, 0.001 Na₂MoO₄.2H₂O, 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 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).

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.

\[ \text{Emulsification index} = \left( \frac{\text{emulsion layer height}}{\text{total height}} \right) \times 100 \]

\[ \text{Emulsification Index} = \frac{\text{Emulsification layer height}}{\text{Total height}} \]

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 sulfurous 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.

\[ \text{Foaming} = \frac{\text{Height of foam}}{\text{Total height}} \times 100 \]

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 K₂HPO₄ and 7.3g/l KH₂PO₄) and diluted using the same buffer solution to an optical density
(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.

\[
\text{% of bacterial cell adherence} = (1 - \left( \frac{\text{OD}_\text{shaken with oil}}{\text{OD}_\text{original}} \right)) \times 100
\]

Where: \( \text{OD}_\text{shaken with oil} \) is the OD of the mixture containing cells and crude oil.

\( \text{OD}_\text{original} \) 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* (8A1), *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*; 8A1 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), 8A1 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

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

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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 42 cm.

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      |
| Lysinibacillus sphaericus | Positive | Positive            | Positive      |
| Pseudomonas sp.    | Positive      | Positive            | Negative      |
| Consortium         | Positive      | Positive            | Negative      |

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

| Isolates     | Foaming activity (cm) | Surface tension (cm) |
|--------------|-----------------------|----------------------|
| Brucella intermedia | 1.63±0.02 a         | 4.00±0.10 a          |
| Pseudomonas aeruginosa | 1.20±0.10 ab       | 4.70±0.10 b          |
| Kocuria indica    | 1.00±0.10 ab         | 4.50±0.10 b          |
| Lysinibacillus sphaericus | 0.33±0.00 a      | 4.20±0.20 a          |
| Pseudomonas sp.   | 1.60±0.10 ab         | 4.00±0.10 a          |
| Consortium       | 2.50±0.10 b          | 4.67±1.53 a          |

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

| Isolates            | Crystal violet Test |
|---------------------|---------------------|
| Kocuria indica      | 0.85±0.03e          |
| 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 45 cm with foaming activity of 1 cm. 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 600 (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 had a low percentage of cells bound to the hydrocarbon phase. For Kocuria indica, the percentage of cell-bound to 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 had a low percentage of cells bound to the hydrocarbon phase.

The C-H bending band of Brucella intermedia was observed in the region 1435 cm⁻¹ with a peak height of 1.2827 cm and 1.1747 cm, respectively. The product also contains residual water vapor, observed in the region 1636 cm⁻¹. Residual water vapor was also observed in the region 1435 cm⁻¹ with a peak height of 1.2827 cm and 1.1747 cm, respectively.
a constituent of the product, which was proved at 1804 cm⁻¹ with a peak height of 5.9052 cm. C-O-C polysaccharides were observed at peak 1127 cm⁻¹ with a height of 8.7747 cm. Benzene ring ortho-substituted and meta-substituted with condensed ring system were observed at the peak of 736 cm⁻¹ and a peak height of 17.7787 cm (Figure 7).

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

The stretching band of *Kocuria indica* was proved from the band at 1742 cm⁻¹ with a peak height of 6.7274 cm. The C-H bending aliphatic band was also observed at peak 1434 cm⁻¹ with a peak height of 6.7639 cm. Residual water vapor was also a constituent of the product, observed at the region 1827 cm⁻¹ with a peak height of 7.0581 cm (Figure 9).

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

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

The stretching band of *Lysinibacillus sphaericus* was proved...
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 biosurfactant-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 500-4000 cm⁻¹. The peak of LD15A (Lysinibacillus sphaericus) in the region of 1137 cm⁻¹ indicates C-O-C stretching in the rhamnose. A similar result was reported by Thenmozhii, et al. (2011). Rhamnolipids produced by Lysinibacillus sphaericus were the most studied biosurfactants 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).

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.

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, Vyta M (2013) Production of a Lipopeptide Biosurfactant by a Novel Bacillus sp. and Its Applicability to Enhanced Oil Recovery. Link: https://bit.ly/2YX08XJ
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/3A9Cqwv

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-alginic beads in the marine environment. Bioresour Technol 280: 88-94. Link: https://bit.ly/3aOxTr

6. Tahhan RA, Ammari TG, Goussous SJ, Al-Shdaifat HI (2011) Enhancing the performance and kinetics of immobilized cells in straw-alginic beads in the marine environment. Bioresour Technol 280: 88-94. Link: https://bit.ly/3aOxTr

7. Glowomofe TO, Olueye 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 Agbagbui 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. Bioinformation 14: 304-314. Link: https://bit.ly/3aMTUhj

10. Nayarisseri A, Sing 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. Mantu 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/2YiKWo

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

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/3jbt1Ft

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/3aQkial

15. Seoud MA, Maachi A, 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/3BSOZJr

16. Amoa JA, Omjosaola PF, Barooah M (2019) Isolation and characterization of some exopolysaccharide producing bacteria from cassava peels heaps. Scientific African 4: e00093. Link: https://bit.ly/3pcAgj9

17. Ruggieri 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/3aQF6eh

19. Tabataee 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/3n11WvS

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/3FWw2aw

21. Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73: 1163-1172. Link: https://bit.ly/3DLN4MM

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

23. Wilson MR, Naccache SN, Samayo 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/3pBF7

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/3pBzXXK

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