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Molecular characterization of biosurfactant-producing bacteria from a crude oil polluted soil in Nigeria

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Abstract

Most biosurfactant-producing microorganisms are hydrocarbon degraders. The research was conducted to isolate and characterize biosurfactant-producing bacteria from a crude oil polluted soil in Nigeria. Biosurfactant-producing bacteria were isolated from crude oil-polluted soil. Crude oil-polluted soil was collected by random sampling and its physicochemical analysis was done. Bacteria were isolated from the contaminated soil and screened for biosurfactant production. Organisms that showed the ability to produce biosurfactant were identified using morphological, biochemical and molecular methods. The physicochemical parameters of the soil showed a pH 6.9, electrical conductivity of 71.5, 2.55% carbon, 2.016% nitrogen and 5.98% phosphorus. The values of biosurfactant tests showed that organisms S2 and S13 were positive for biosurfactant production. The percentage emulsion indexes of the two selected organisms S2 and S13 were 59.09% and 57.14% respectively. The Blast analysis from the molecular identification showed that the isolated organisms were *Gordonia alkanivorans* for S2 and *Tsukamurella inochensis* for S13. This research showed that the isolated biosurfactant-producing bacteria are abundant in the crude oil polluted soil.

Key words: Biosurfactant, Crude oil, *Gordonia alkanivorans, Tsukamurella inochensis,* soil.

Introduction

Crude oil, also known as petroleum, is an unrefined natural petroleum product consisting of deposits of hydrocarbons and other organic materials (Chen, 2023). Petroleum hydrocarbons are major environmental pollutants generated from coastal oil production, transportation, refining, shipping operations, offshore oil production, and large-scale oil spills (Arulazhagan et al., 2010). Human activities such as municipal run-offs and liquid releases from industries cause crude oil pollution, which impacts the environment and poses a direct or indirect health hazard to forms of life (Sajna et al., 2015). In the soil, certain bacteria have a special ability to break down or convert organic pollutants into harmless biological products. Bioremediation practice is mainly based on the use of these talented microorganisms that exist in the soil (Esin and Ayten, 2011).

Bacteria and fungi utilize petroleum hydrocarbons as a source of carbon. Fungi such as *Aspergillus, Penicillium, Fusarium, Amorphotheca, Neosartorya, Paecilomyces, Talaromyces, Graphium Cunninghamella,* and bacteria such as *Pseudomonas, Gordonia, Tsukamurella, Mycobacterium, and Sphingomonas* are microorganisms capable of degrading difficult pollutants (Eman and Andrew, 2017; Didiugwu, 2021).

The presence of high molecular weights and very poorly water-soluble compounds prevents natural biodegradation from working effectively in hydrocarbon-contaminated soils (Didiugwu, 2021).

In general, petroleum hydrocarbon compounds bind to soil components and are difficult to remove or decompose (Nilanjana and Preethy, 2011). Biosurfactants produced mainly by microorganisms are biologically active surfactants that can help remove or degrade these petroleum hydrocarbons (Ambaye et al., 2021; Didiugwu, 2021). Biosurfactants have surface activity, high tolerance to various environmental factors, and tolerance to extreme conditions such as acidity or basicity of aqueous solutions, temperature, concentration of salt and ionic strength. It also has biodegradability, demulsifying-emulsifying ability, and anti-inflammatory and antibacterial activity (Peele, 2017).

The different properties of surfactants are dispersing, emulsifying or demulsifying, wetting, foaming, and coating, and therefore they are very useful in biological and physico-chemical treatment technology for metal and organic pollutants (Wu & Lu, 2015). There are different classes of biosurfactants based on their properties, antimicrobial activity, efficiency in the production and removal of hydrocarbons from the environment, and their ability to reduce surface tension. (Tabatabaei, 2015).

Biosurfactants accelerate the biodegradation of organic compounds by increasing their solubility through emulsification. Most crude oil-degrading bacteria release extracellular biosurfactants to facilitate oil absorption and degradation of hydrocarbons. This study aimed to isolate and characterize biosurfactant-producing bacteria from a crude oil-polluted soil in Nigeria.

MATERIALS AND METHODS

Description of Sampling Area

The Ibeno Local Government Area covers a coastal area of more than 1,200 square kilometers. It is located on the eastern shore of the Niger Delta, which is part of the Gulf of Guinea. It is located at the southern tip of Akwa Ibom State at latitudes 4°321 and 4°341 north of the Equator and longitudes $7^0$541 and 8°021 east of the Greenwich Meridian. The region is characterized by a humid tropical climate with an annual rainfall of 4021 mm, an average relative humidity of 80%, and an average minimum and maximum temperature of 22°C and 30°C.

Sampling Site

The soil for the isolation of biosurfactant-producing bacteria was collected using a sterile steel spatula and zip-locked bag from an oil drilling site in Ibeno, Akwa Ibom State at a depth of 5cm. Composite sampling was used in collecting the soil samples. The soil samples were pooled separately, labeled, packaged with ice, and immediately transported to the Godfrey Okoye University Microbiology Laboratory for analysis as described by (Adieze, 2012).
Qua Iboe Brent crude oil was collected from Frontier Oil Company at Qua Iboe Terminal, Ibeno Local Government Area, Akwa Ibom State. Qua Iboe Brent was used in all studies.

Oil sterilization

Crude oil was sterilized with a micron Chromafil CA/S %45 syringe filter according to the method described by Chorom et al. (2010).

Soil Physicochemical Analysis

Physicochemical characteristics of contaminated soils were determined using the mentioned methods. Nitrogen was determined using the method of AOAC (1990). pH, conductivity, salinity and phosphorus (APHA, 1998). Total organic carbon (ASTM, 1995a). The particle size of the soil was carried out following ASTM (1995b) method. Mercury, arsenic, aluminum, selenium, molybdenum, and tin (APHA, 1995). Water holding capacity was done using a method described in Notes for free.com (2017). Cation exchange capacity (CEC), Iron, copper, zinc, cadmium, nickel, lead, chromium, manganese, cobalt, vanadium, and silver were also determined using ASTM (1999) and APHA (1998) methods.

Bacterial Isolation

Bacteria were isolated from crude oil-polluted soil using the enrichment culture technique described by Liu et al. (2010). Two hundred and fifty ml Erlenmeyer flasks (DWK, UK) containing 100 ml of sterile medium consisting of mineral salts including NaCl (30 g/L), $K_2HPO_4.3H_2O$ (1.0 g/L), KH_2PO_4 (1.0 g/L), NH_4NO_3 (1.0 g/L), $MgSO_4.7H_2O$ (0.2 g/L), $CaCl_2.2H_2O$ (0.02 g/L),

and $FeCl_3$ (0.05 g/L), 0.2 ml of crude oil, and 1g of contaminated soil were incubated in a rotary shaker (Stuart, England-UK) at 130 rpm at 30°C for 10 days. Five ml of inoculum was transferred from the enriched medium to a new Mineral Salt Medium (MSM) and incubated for another 10 days cycle. One ml of culture medium was serially diluted in 9 ml sterile saline solution (0.85% NaCl) (AnalaR, England) after six successive transfers. One hundred (100) µL of the appropriate dilution was poured onto sterile MSM agar plates. Crude oil was added to MSM and stirred well before dispensing into plates. These were then incubated at room temperature for 7 days. Pure colonies were subcultured and stored in nutrient agar (TM media, India) slants at 4°C for further characterization and analysis.

Screening for Biosurfactant

The resulting colonies were tested for biosurfactant production by different methods. These include oil displacement, drop collapse, hemolysis, and emulsification tests.

Oil displacement: The oil displacement test was developed by Morikawa et al. (2000). Ten (10) ul of crude oil were added to the surface of 40 ml of distilled water in a Petri dish to form a thin layer of oil. Then, 10 ul of culture supernatant were gently placed in the centre of the oil layer. The presence of biosurfactant in the supernatant displaced the oil and formed a clean zone. The clear zone was measured with a vernier caliper and placed on a meter rule to take the reading.

Drop collapse test: crude oil was used in this test. Two microlitres of oil were applied to the well area delimited on the covers of 96-well microplates and these were left to equilibrate for 24 h. Five microliters of the 72 h culture after centrifugation at 4000rev for 15 min to remove cells, were transferred to the oil-coated well regions, and drop size was observed after 1 min with the aid of a magnifying glass, as done by Saravanan and Vijayakumar (2012). The result was considered positive for biosurfactant production when the drop was flat, and those cultures that gave rounded drops were scored as negative, indicative of the lack of biosurfactant production as described by Youssef et al. (2004).

Emulsification test (E24): This was carried out as done by Bodour et al. (2004). Several colonies of pure culture were suspended in test tubes containing 2 ml of mineral salt medium and after 48 h of incubation, 2 ml of hydrocarbon (oil) were added to each culture broth tube. Then, the mixtures were vortexed at 3200 rpm for 1 min and allowed to stand for 24 h. The emulsion index (E24) is the height of the emulsion layer (cm) divided by total height (cm), multiplied by 100.

$$\text{Emulsification index} = \frac{\text{Height of the emulsion layer}}{\text{Total height}} \times 100$$

Haemolysis activity: A pure culture of bacterial isolates was streaked on the freshly prepared blood agar and incubated at 37°C for 48 h, as described by Saravanan and Vijayakumar (2012). Positive strains caused lysis of the blood cells and exhibited a colorless, transparent ring around the colonies.

Characterization and Identification of Isolates

The isolated organisms were characterized by morphological, biochemical, and molecular methods.

Morphological Characterization

Colonial characteristics

Pigmentation and morphological properties such as colour, texture, shape, and elevation characteristics were observed after incubation.

Gram staining

This was carried out as done by Rollins and Joseph (2000). This technique divides bacterial species into Gram-positive and Gram-negative groups. A smear of the culture was made on a clean, dry grease-free glass slide using a sterile wire loop. The smear was air-dried and heat-fixed by passing over the Bunsen burner flame three times. After cooling, the slide was flooded with 0.5% crystal violet solution and left for a minute. It was washed off with water and then flooded with lugol iodine (which served as a mordant that fixes the dye inside the cell). The iodine was washed off after one minute, and

95% ethanol was used to decolourize the smear for 10 seconds. The smear was counter-stained with dilute safranin (0.25%) dye for 30 seconds. It was then washed off and the slide air-dried. A drop of immersion oil was placed on it and observed under the microscope using an oil immersion objective lens. Gram positive and negative reactions were indicated by blue and red colours respectively.

Biochemical Characterization

Pure colonies of the bacterial isolates were characterized and identified using Bergey's Manual of Determinative Bacteriology, authored by Holt et al. (1994). Several biochemical tests were performed to aid in the identification. They were motility test, catalase test, citrate test, Indole test, methyl red, urease test, hydrogen sulphide production test and sugar fermentation test.

Motility test

The Cheesbrough (2000) method was applied. A directional and purposeful movement of the organisms demonstrates motility. 0.2% agar (HKM, Guangdong) was added to the nutrient broth before it was poured into test tubes and autoclaved for 15 minutes at 121^0C and 15psi. Test tubes that had been inoculated were incubated for 24 hours. Motility was shown by diffused growth, which extended throughout the media. The growth of non-motile organisms followed the incubation line.

Catalase test

The Cheesbrough (2000) method was applied. By using this test, organisms that produce the enzyme catalase can be distinguished from those that do not. On a clean slide, a drop of 30% freshly made hydrogen peroxide (3 milliliters H_20_2 in 7 milliliters H_20) was added. It was filled with a loopful of isolate and emulsified. A positive test resulted from the development of gas bubbles, or effervescence, which suggested the existence of catalase. The absence of bubbles suggested that the response was negative.

Citrate test

The Aryal (2019) method was applied. The purpose of the test was to identify the organisms that could use citrate as their only source of carbon for metabolism. Simmon's Citrate Agar (Accumix, India) slants were made in compliance with the manufacturer's guidelines. A loopful of an 18-hour-old culture was streaked over the surface of the slants to inoculate them, and they were then incubated for 48 hours at 37^0C. A shift in color from green to blue signified a positive outcome, whereas a lack of color change suggested a poor outcome.

Indole test

The Cheesbrough (2000) method was applied. After the tryptone-broth was dissolved in water, 5 milliliters were added to each tube and incubated. The isolates were inoculated into the tube and incubated for 48 hours at 28^0C. The tubes were filled with five drops of Kovac's reagent (4 p-dimethy-aminobenzaldhyde), gently shaken, and then allowed to settle. An outcome that was positive was shown by the alcohol dye turning red.

Methyl red

The McDevitt (2009) method was applied. Sterile tubes containing buffered glucose peptone broth (consisting of 1.5% peptone water, 0.5% glucose, and 0.5% dipotassium phosphate) were inoculated and incubated for 48 hours at 37°C. Next, 5 milliliters of each culture were mixed with 5 drops of methyl red reagent. Positive results were shown by the brilliant red color that appeared as soon as the reagent was added.

Urease test

The Tankeshwar (2012) approach was applied. The urease test medium was produced in accordance with the manufacturer's instructions (Himedia). In a conical flask, 24g of urease agar was dissolved in 950 ml of distilled water. It was autoclaved for 10 minutes at 115^0C. Following a 45°C cooling period, 50 ml of 40% filter-sterilized urea was added, mixed, and then poured into test tubes. After being slanted, the tubes were left to cool. A loopful of a 48-hour-old culture was streaked over the surface of the slants to inoculate them, and they were then incubated for 48 hours at 30^0C. A shift in color from yellow to pink signified a positive outcome,

whereas an absence of color change suggested a negative result.

Hydrogen-sulphide production test

The method of Aryal (2019) was used. Hydrogen sulfide (H2S) production tests are used to detect hydrogen sulfide gas produced by living organisms. Peptone water (Accumix, India) was prepared according to the manufacturer's instructions. It was inoculated with the microorganisms. A piece of lead-acetate paper was inserted into the neck of the test tube above the medium and sealed tightly. It was then incubated at 30 °C and inspected daily for blackening at the bottom of the strip, which indicates a positive reaction for gas production.

Sugar fermentation test

The method of Aryal (2020) was used. This test measured the ability of isolates to ferment glucose, lactose, sorbitol, fructose, sucrose, mannose, and galactose to produce acids and gases. The fermentation medium consisted of 1% peptone water, 5 drops of 0.2% bromothymol blue indicator solution, and 1% sugar granules. 9 ml of medium was placed in a clean and dry test tube. Durham tubes were placed into it (inverted, without headspace) and sterilized by autoclaving at 115 °C for 15 min. After cooling, a loopful of test microorganisms was inoculated and incubated at 30°C for 24 hours. A positive reaction was recorded as a color change of the medium from blue to yellow, while the appearance of an empty space at the end of the Durham tube indicated gas formation.

Molecular Characterization

Molecular characterization of the bacterial isolates was done at the laboratory of the University of Illinois at Chicago Sequencing Core (UICSCQ). The molecular characterization involved DNA extraction for bacterial isolates stored in a DNA/RNA shield, polymerase chain reaction (PCR) amplification, and Sanger Sequencing.

Sanger sequencing of bacterial isolates

Genomic DNA from bacterial cells was extracted using an automated DNA extraction device, the Maxwell16 instrument (Promega), implementing the Maxwell® 16 Tissue DNA Purification Kit (Promega, USA) according to the manufacturer's protocol. Genomic DNA was subjected to PCR amplification with the primer set 27F (AGAGTTTGATCMTGGCTCAG) / 1492R (GGTTACCTTGTTACGACTT) using DreamTaq Green PCR Master Mix (2X) (ThermoFisher, USA). PCR-amplified DNA was purified with AMPure XP beads (0.6X) (Beckman Coulter, USA) to remove unused primers and dNTPs. Amplified genomic DNA was sequenced on an ABI 3730xl capillary sequencer (Thermofisher, USA) using the 27F primer to initiate the sequencing reaction. Sequence data was trimmed to remove poor quality bases, and sequence analyses were performed using the NCBI BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the SILVA on-line aligner (https://www.arb-silva.de/aligner/), and the software package MEGA (https://www.megasoftware.net/).

RESULTS AND DISCUSSIONS

The physicochemical properties of the polluted soil sample are shown in Table 1. Some of the analyzed properties, like Odour and texture, showed that the soil contained crude oil. The Odour was that of a crude oil smell while the soil texture was loose; a good soil sample was supposed to be bound.

Table 1: Physicochemical properties of the polluted soil sample for microbial Isolation

Parameters	Value
pH	6.9
Electrical conductivity,us/cm	71.5
Salinity, ppt	0.1987
Cation Exchange Capacity ,cmol/kg	24.4803
Vanadium, mg/kg	0.385
Lead, mg/kg	0.712
Chromium, mg/kg	0.536

Zinc, mg/kg	13.533
Cadmium, mg/kg	0.002
Iron, mg/kg	208.38
Manganese, mg/kg	5.738
Copper, mg/kg	0.590
Cobalt, mg/kg	0.00
Silver, mg/kg	0.008
Selenium, mg/kg	0.523
Molybdenium, mg/kg	0.098
Aluminium, mg/kg	0.244
Tin, mg/kg	0.00
Mercury, ppm	0.039
Arsenic, ppm	0.284
Nitrogen, %	2.016
Carbon,%	2.55
Phosphorus, %	5.98
Sand,%	88.26
Silt, %	5.4
Clay,%	6.34
Odour	Crude oil
Texture	Loose

Table 1 result positively correlated with the findings of Ogbonna and Amajuoyi (2009) when measuring the physicochemical properties of crude oil-contaminated area and reporting that hydrocarbon pollution makes the parameter values higher than the specified limit.

In biosurfactant testing, more emphasis has been placed on strains of biosurfactant-producing bacteria that can facilitate biodegradation and bioremediation. Some of the isolates were positive for the haemolysis test. Only S2 (*Gordonia alkanivorans)* and S13 (*Tsukamurella inochensis)* were positive in the drop collapse and oil displacement tests (Table 2).

Table 2: The values of biosurfactant screening parameters

Organism sample	Haemolysis test	Drop collapse	Oil displacement	Emulsion index (%)
S1	+	-	-	0
S2	+	+	+	59.09
S3	-	-	-	7.69
S4	-	-	-	0
S5	+	-	-	0
S6	+	-	-	7.41
S7	-	-	-	10
S8	-	-	-	7.14
S9	+	-	-	17.24
S10	+	-	-	3.57
S11	-	-	-	0
S12	+	-	-	0
S13	+	+	+	57.14

During incubation, crude oil emulsification was evident in their cultures, indicating extracellular biosurfactant/bio-emulsifier production. The isolates emulsified the crude oil after only 5 days of incubation; when left to stand, a thin layer was developed, which was dispersed by gentle stirring. In

contrast, the oil layer from the other flasks remained on the surface after another 5 days. Both isolates had a high emulsion index of 59.09 for *Gordonia* sp. and 57.14 for *Tsukamurella* sp. Five different methods were used as Zeena et al. (2023) reported that more than one method should be included in the preliminary selection to accurately identify potential biosurfactant producers. Some isolates that test positive for hemolysis but are negative in other tests may not be biosurfactant-producing strains. Ghasemi et al. (2019) reported that the hemolytic activity must be considered a preliminary test and an unreliable criterion to detect the presence of any BS molecule in a microbial culture. The results of the biosurfactant screening were positively correlated with the findings of Kugler et al. (2014), who stated that *Tsukamurella* sp could produce the lipid surfactant trehalose. In addition, Franzetti et al. (2009) and Ta-chen et al. (2008) also reported the production of biosurfactant by *Gordonia* sp.

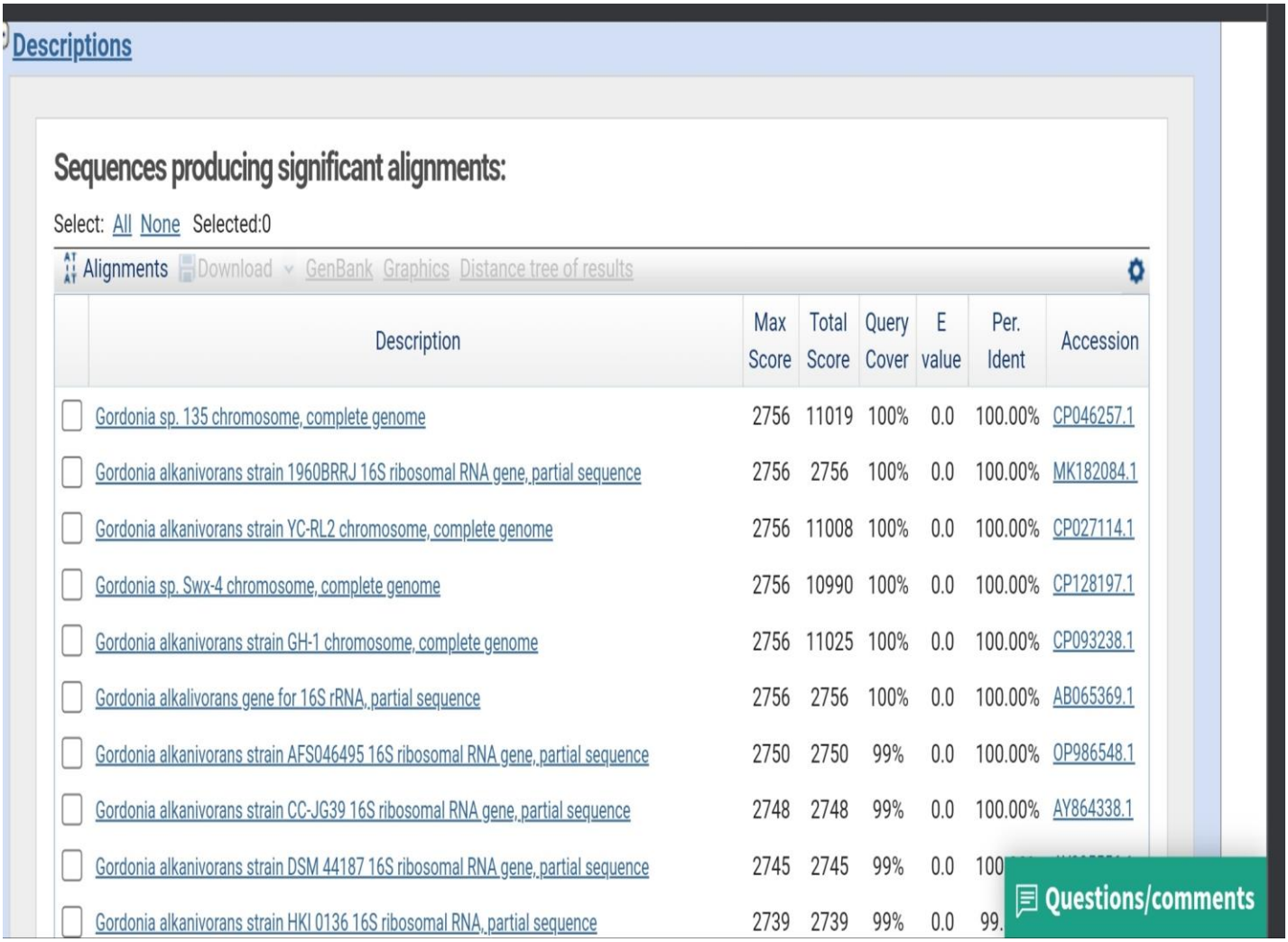
Table 3: Morphological and biochemical characterization of isolated bacteria

	S2	S13
Colour	Pink	Cream
Texture	Smooth	Dry/rough
Shape	Circular	Irregular
Elevation	Raised	Flat
Gram reaction	+ve	+ve
Microscopic appearance	Small cocci in chains	Bacilli appearing singly
Citrate	-	+
Indole	-	-
Methyl red	-	-
Catalase	+	+
Oxidase	-	-
Hydrogen sulphide	-	+
Urease	-	+
Motility	-	-
Sucrose	-	+
Fructose	+	+
Glucose	-	+
Lactose	-	+
Mannose	-	-
Galactose	-	-
Sorbitol	-	-
	Gordonia sp	*Tsukamurella sp*

+ = positive, - = negative

Two bacterial strains identified and used in the study were *Gordonia alkanivorans and Tsukamurella inochensis* strain Yaoman (JQ806393.1). The morphological and biochemical properties (Table 6) were consistent with those of the named isolates and were

positively correlated with molecular identification after sequence analysis (Figures 1 and 2).



Descriptions

Sequences producing significant alignments:

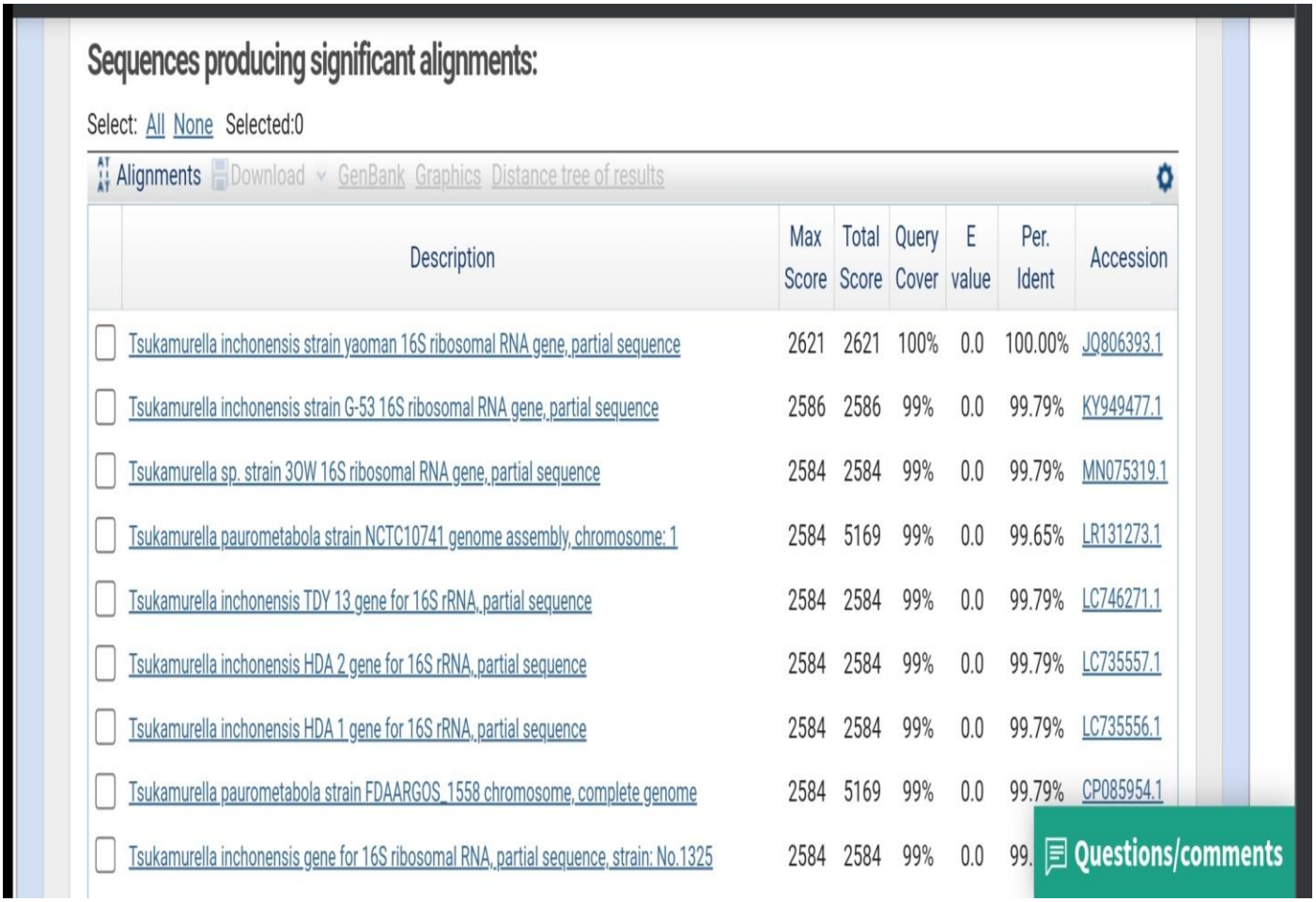
Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☐	Gordonia sp. 135 chromosome, complete genome	2756	11019	100%	0.0	100.00%	CP046257.1
☐	Gordonia alkanivorans strain 1960BRRJ 16S ribosomal RNA gene, partial sequence	2756	2756	100%	0.0	100.00%	MK182084.1
☐	Gordonia alkanivorans strain YC-RL2 chromosome, complete genome	2756	11008	100%	0.0	100.00%	CP027114.1
☐	Gordonia sp. Swx-4 chromosome, complete genome	2756	10990	100%	0.0	100.00%	CP128197.1
☐	Gordonia alkanivorans strain GH-1 chromosome, complete genome	2756	11025	100%	0.0	100.00%	CP093238.1
☐	Gordonia alkalivorans gene for 16S rRNA, partial sequence	2756	2756	100%	0.0	100.00%	AB065369.1
☐	Gordonia alkanivorans strain AFS046495 16S ribosomal RNA gene, partial sequence	2750	2750	99%	0.0	100.00%	OP986548.1
☐	Gordonia alkanivorans strain CC-JG39 16S ribosomal RNA gene, partial sequence	2748	2748	99%	0.0	100.00%	AY864338.1
☐	Gordonia alkanivorans strain DSM 44187 16S ribosomal RNA gene, partial sequence	2745	2745	99%	0.0	100	
☐	Gordonia alkanivorans strain HKI 0136 16S ribosomal RNA, partial sequence	2739	2739	99%	0.0	99.	

Questions/comments

Figure 1: The blast result of isolate S2 (*Gordonia alkanivorans*)



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tsukamurella inchonensis strain yaoman 16S ribosomal RNA gene, partial sequence	2621	2621	100%	0.0	100.00%	JQ806393.1
Tsukamurella inchonensis strain G-53 16S ribosomal RNA gene, partial sequence	2586	2586	99%	0.0	99.79%	KY949477.1
Tsukamurella sp. strain 30W 16S ribosomal RNA gene, partial sequence	2584	2584	99%	0.0	99.79%	MN075319.1
Tsukamurella paurometabola strain NCTC10741 genome assembly, chromosome: 1	2584	5169	99%	0.0	99.65%	LR131273.1
Tsukamurella inchonensis TDY 13 gene for 16S rRNA, partial sequence	2584	2584	99%	0.0	99.79%	LC746271.1
Tsukamurella inchonensis HDA 2 gene for 16S rRNA, partial sequence	2584	2584	99%	0.0	99.79%	LC735557.1
Tsukamurella inchonensis HDA 1 gene for 16S rRNA, partial sequence	2584	2584	99%	0.0	99.79%	LC735556.1
Tsukamurella paurometabola strain FDAARGOS_1558 chromosome, complete genome	2584	5169	99%	0.0	99.79%	CP085954.1
Tsukamurella inchonensis gene for 16S ribosomal RNA, partial sequence, strain: No.1325	2584	2584	99%	0.0	99.	

Questions/comments

Fig 2: The blast result of isolate S13 (*Tsukamurella inochensis* strain yaoman)

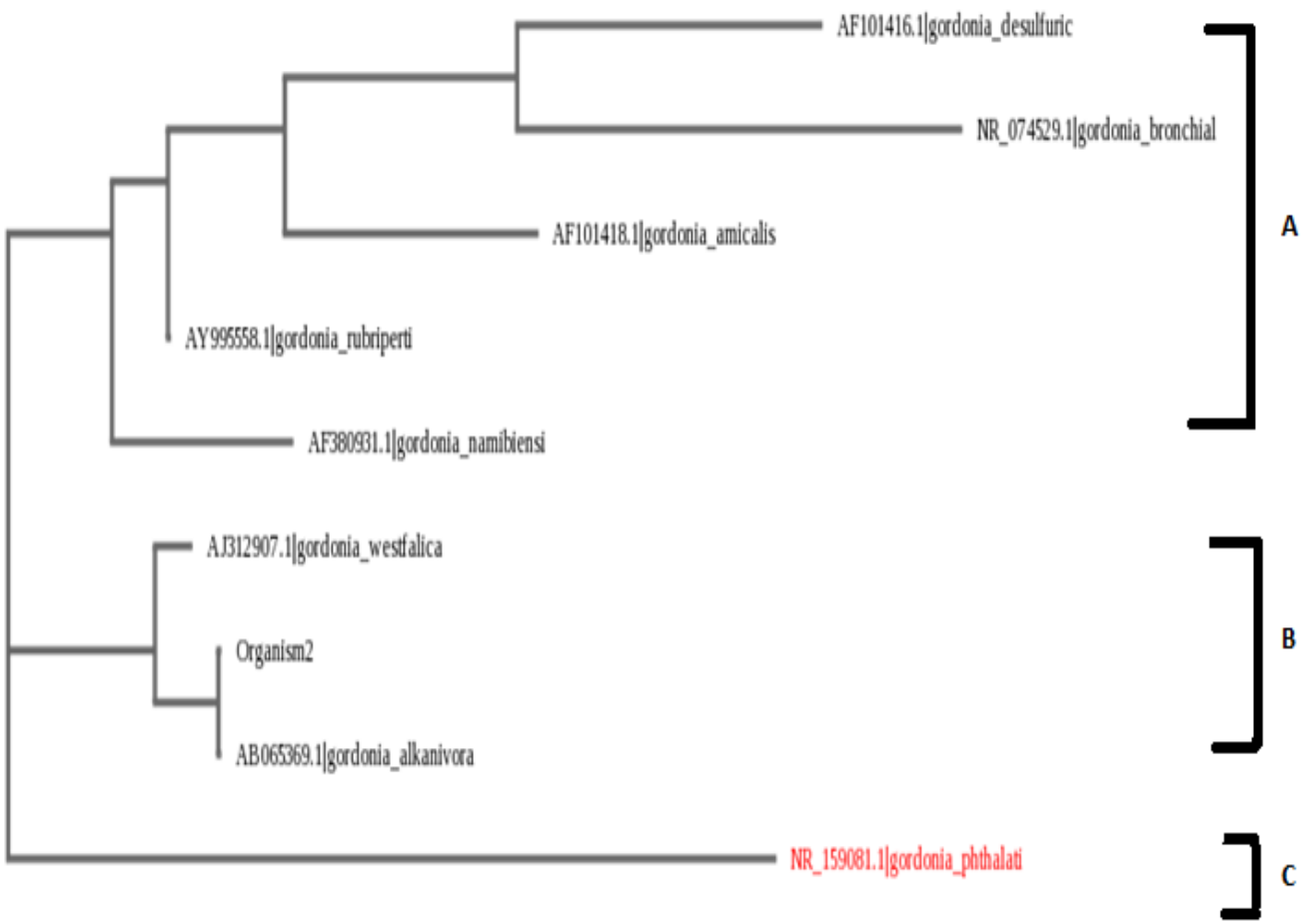


Fig 3: Phylogeny tree of *Gordonia alkanivorans*

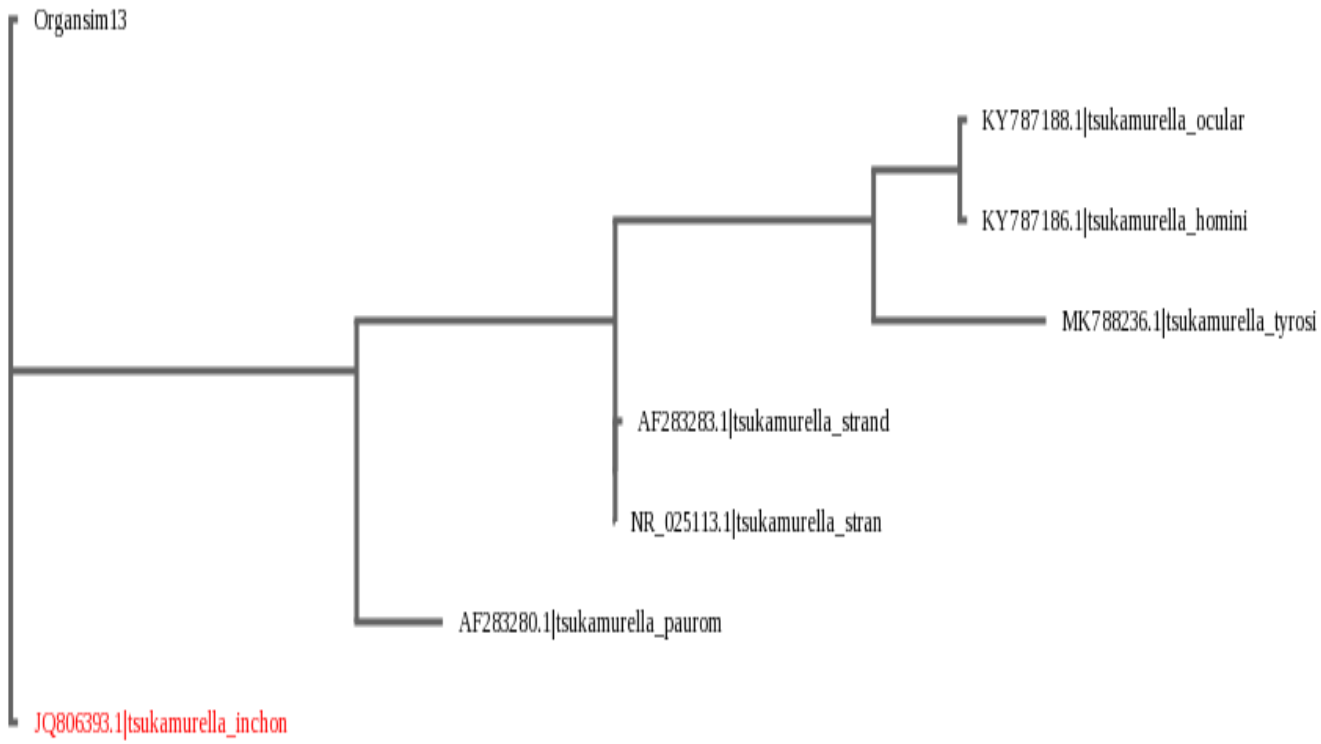


Fig 4: Phylogeny tree of *Tsukamurella inochensis*

The result of the phylogeny tree in figure 3 showed that it has three major groups or clusters (A, B, and C). Organisms in a cluster are closely related. The organism in Cluster A is distantly related to Cluster C. Among the organisms in Clusters A and B, Cluster C is an outlier. *Gordonia alkanivorans* belongs to cluster B and is more closely related to *Gordonia westfalica*

The phylogeny tree in Figure 4 showed that *Tsukamurella inchonensis* is an outliner. The organisms in the second cluster are closely related, but *T. ocular and T.homini* are more closely related.

CONCLUSION

Biosurfactant-producing microorganisms can be isolated from a crude oil-polluted soil. Their presence in the oil-polluted soil shows that they are hydrocarbon degraders, and the biosurfactant aids in biodegradation by emulsifying hydrocarbons like crude oil.

Further research needs to be done to determine the toxicity level of this biosurfactant. This is necessary because, since biosurfactants can aid in biodegradation, there is a tendency for them to be released into the environment during biodegradation. The ability of the biosurfactant to help in carrying out biodegradation and bioremediation also needs to be accessed.

APPRECIATION

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