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Screening and Characterization of Lipopeptide Biosurfactant Producing Bacteria from Various Natural Sources

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Abstract: ***Surface-active chemicals, secreted extracellular by microorganisms are known as biosurfactants. In present study, biosurfactant production and typically lipopeptide surfactant production was studied. By various screening procedures five potent biosurfactant-producing strains (PS1, PS2, M1, M2, and L1) from natural sources were identified. Biosurfactant characterization was done by Fourier Transform Infrared Spectroscopy. In the FTIR spectra of carbonyl groups, peptides, alkyl bonds, N-H and C-H stretching vibrations were observed that indicated the presence of lipopeptides. Further partial molecular identification by 16S rRNA gene sequencing was made for most potential strain-PS1. The isolate was identified as a novel Klebsiella quasipnemoniae subsp. similipnemoniae strain (GenBank accession number: ON384439). To determine potential applications of our biosurfactant, we studied its diverse activities, such as antibacterial activity, foaming activity, and plant growth promoting activities.***

Keywords: Biosurfactant, Lipopeptide, FTIR, antibacterial activity

I. INTRODUCTION

Biosurfactants are surface-active molecules with hydrophobic and hydrophilic components that can combine two immiscible compounds such as water and oil by lowering surface tension. Because of their amphipathic nature, biosurfactants also partition at the interface between aqueous and hydrophobic phases, such as oil and water or oil and rock. Because of their increased biodegradability, environmental acceptability, lower toxicity, and lower CMC, biosurfactants have been proposed as a replacement for chemically generated and conventional surfactants.

Microbial surfactants are also more stable and effective across a broader range of environmental conditions, including temperature, pH, and salinity[1]. As a result, biosurfactants appear to be more promising than synthetic surfactants for environmental oil recovery methods. Lipopeptides are composed of a hydrophilic (four to twelve amino acid) moiety linked to a hydrophobic moiety (β-hydroxy fatty acids). Biosurfactants, more specifically 'Lipopeptides,' are short chains of amino acid monomers joined together by peptide (amide) bonds. Based on a survey in the database NORINE[2]. identified 263 distinct lipopeptides produced by 11 microbial taxa. The most abundant lipopeptide producers are *Pseudomonas spp., Streptomyces spp., and Bacillus spp.* with 78,40, and 98 different lipopeptides, respectively, classified into 11, 5, and 6 lipopeptide families.

Certain bacteria have been discovered to produce lipopeptides. As a result, biosurfactants are thought to be more promising than synthetic surfactants for environmental oil recovery methods and are expected to replace them. Lipopeptides contain a hydrophilic moiety of 4 to 12 amino acids connected to a hydrophobic moiety of β-hydroxy fatty acids. Biosurfactants, more specifically "Lipopeptides," are short chains of amino acid monomers linked together by peptide (amide) bonds[3]. Lipopeptides are most commonly produced by *Bacillus, Brevibacillus, Streptomyces, Arthrobacter, and Pseudomonas*, though *Klebsiella, Rhodococcus, and Aspergillus* have also been reported to produce lipopeptides.

Lipopeptide biosurfactants have antifungal, antibacterial, antiviral, haemolytic, and anti-cancer properties. Lipopeptides are secondary metabolite products produced by the secondary metabolic pathway of bacteria. There are two ways to produce lipopeptide: cyclic lipopeptide (Non-Ribosomal pathway) and non-cyclic lipopeptide/linear lipopeptide (Ribosomal pathway)[4]. Lipopeptides have the ability to emulsify and de-emulsify, moisturise and disperse, anti-wrinkle, anti-larvicide, viscosity reducers, lipopeptide absorption capacity, anti-adhesive, food preservative, flotting agent and corrosion inhibitors, and anti-inflammatory. It is used in a variety of industries including pharmaceuticals, food, foaming agents in detergents, laundry detergents, agricultural, medical, and cosmetics[5].

The purpose of this study was to screen and characterize lipopeptide biosurfactants produced by isolates included under study.

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II. MATERIAL AND METHODS

A. Samples Collection

In present study, in March 2022, total 6 samples were collected to isolate biosurfactant producers. Petroleum-contaminated soil was collected from two different locations in Surat. The top layer (0-15 cm) of surface soil was collected with a sterile spatula and placed in sterile zip-locked bags. 2 marine water samples were collected from the beaches of Dumas and Hazira and the fermented milk products (paneer whey and curd whey) were collected from various locations in Surat, Gujarat [6,7,8]

B. Enrichment and Isolation

One gram of each soil sample was dissolved in 10 mL sterile water and then streak into enrichment medium (BHM) agar. $MgS0_4$-0.2g/L, $CaCl_2$-0.02g/L, KH_2PO_4-1g/L, K_2HPO_4-1g/L, NH_4NO_3-1g/L, and $FeCl_3$-0.05g/L were all found in BHM (per litre). pH adjusted to 7.0 (as the sole carbon source) and incubated for one week at 37°C. Single colonies from the plate were chosen and streaked on fresh plates until pure cultures appeared, which were maintained as slant cultures, after incubation at 37°C for 7 days [6]. One millilitre of each marine water sample was mixed with ten millilitres of sterile water before being poured into the enrichment medium, Starch casein agar (SCA) plate. SCA content (per litre) Soluble starch: 10.0g/L, Casein: 0.30g/L, KNO_3: 2.0g/L, $MgSO_4.7H_2O$: 0.05g/L, K_2HPO_4: 2.0g/L, NaCl: 2.00g/L, $CaCO_3$: 0.02g/L, $FeSO_4.7H_2O$: 0.01g/L, Agar: 18.0. pH adjusted to 7.0 and incubated for 3 days at 37°C. Following incubation, single colonies from the plate were picked and streaked on fresh plates repeatedly until pure cultures appeared, which were then preserved as slant cultures [7]. The fermented milk products were streaked on the De Mann Rogosa Sharpe (MRS) agar plate. MRS (per litre) contained Protease peptone - 10.00 g/L, Beef extract - 10.00 g/L, Yeast extract - 5.00 g/L, Dextrose - 20.00 g/L, Tween 80 - 1.00 g/L, Ammonium citrate - 2.00 g/L, Sodium acetate - 5.00 g/L, Magnesium sulphate - 0.100 g/L, Manganese sulphate 0.050, Dipotassium phosphate - 2.00 g/L, Calcium carbonate - 10 g/L, Agar - 30 g/L pH adjusted to 6.50.2 and incubated at 37°C for 2 days. Following incubation, single colonies from the plate were picked and streaked on fresh plates until pure cultures appeared, which were then preserved as slant cultures [8].

C. Morphological and biochemical characterization of isolates

The selected isolates were characterised by determining colony morphology, biochemical and growth characteristics, and fermentation of various carbohydrates [9].

D. Screening of biosurfactant producing bacteria

Biosurfactant production capacity was screened by various methods, such as,

1) *Blood Agar Plate Assay:* Fresh cultures were streaked on blood agar plates containing 5-7% sheep blood to perform the blood agar assay. A favourable result was the formation of a clear halo enclosing the colonies after 48-72 hours of incubation at 37°C [10].
2) *Blue Agar Plate Assay:* Using Siegmund and Wagner's technique, isolated strains were screened on blue agar plates. Each isolated colony has been grown in mineral agar medium aided with methylene blue and cetyltrimetilammonium bromide (CTAB: 0.5 percent mL) (0.2 percent mL). The presence of a dark blue halo around the culture was considered a sign of biosurfactant production [11].
3) *Lipase-activity Test:* Lipase-producing isolates were screened using tributyrin agar plates. 0.1 N NaOH was used to adjust the pH of the medium to 7.3-7.4. On the tributyrin agar plates, a loopful of inoculum was streaked. For 7 days, the plates were incubated at 37° C. The plates were examined after incubation for the formation of a clear zone around the colonies [12].
4) *Drop Collapse Assay:* The drop collapse assay was performed according to using cell-free supernatant prepared by centrifuging a 48-hour culture at 5000 r.p.m. for 20 minutes at 4°C. A single drop of engine oil was placed on a glass slide, followed by one drop of supernatant. After 1-2 minutes, the flattening property was recorded. If the drop collapsed, the result was considered positive; if it remained beaded, the result was considered negative [13].

E. Preliminary Identification of Biosurfactant:

1) *Biuret Test:* The crude extract solution was heated to 70°C before being combined with a 1 M NaOH solution. A change in colour was detected after progressively adding drops of 1% CuSO4. The reaction of peptide bond proteins or short-chain polypeptides produces a violet colour, which indicates a favourable result. Lipopeptide biosurfactant was detected using this test[14].

F. Determination of Biosurfactant Production capabilities

A single isolated bacterial colony was inoculated into production medium and incubated for 6-7 days in to rotary shaker at 37°C and 180 rpm. Biosurfactant production capabilities was checked by,

1) *Oil Displacement Assay:* The assay was carried out according to the method described by Morikawa et al., 2000. On the surface of the Petri dish, 20 ml of distilled water was mixed with 20 µl of crude oil. On the Petri plate, 10 µl of cell-free culture broth was added to the oil surface. The presence of biosurfactant in the cell-free culture broth displaced the oil around it, forming an oil-free clearing zone whose diameter indicates surfactant activity, which was referred to as oil displacement activity. As a negative control, distilled water was used [15].
2) *Emulsification Index Assay:* As previously reported, the emulsification index (E24) was calculated. To the same amount of cell-free supernatant, three microlitres of kerosene was added and vortexed for two minutes. The height of the stable emulsion layer was measured after 24 hours. The negative control was water [16]. The percentage of the height of the emulsified layer divided by the entire height of the liquid column was calculated as E24: E24 (%) = total height of the emulsified layer/total height of the liquid layer x 100

3) *Bacterial Adhesion to Hydrocarbon (BATH) Assay:* Bacterial adherence to hydrocarbons was used to determine cell hydrophobicity, as described by Rosenberg et al, 1980. The cell pellets were washed twice before being suspended in a buffer salt solution (g/L, 16.9 K_2HPO_4 and 7.3 KH_2PO_4) and diluted to an optical density (OD) of 0.5 at 610 nm using the same buffer solution. 100 µl of crude oil was added to the cell suspension (2 ml) in test tubes (10 ml volume with 10 x 100 mm dimension) and vortex-shaken for 3 minutes. After shaking, the crude oil and aqueous phases were separated for one hour. The OD of the aqueous phase was then measured in a spectrophotometer at 610 nm. From the OD values, percentage of cells attached to crude oil was calculated using the following formula: % Of bacterial cell adherence = (1-(OD shaken with oil/OD original)) x 100

Where: OD shaken with oil - OD of the mixture containing cells and crude oil [17].

4) *Phenol Sulfuric Acid Method:* After incubation, the production broth was centrifuged for 15 minutes at 10,000 rpm, with the supernatant collected and the pellet discarded. 1 ml of the recovered supernatant was combined with 1 ml of 5% phenol, then 5 ml of concentrated H2SO4 was added drop by drop. A yellow to orange colour is produced when biosurfactant is present in the supernatant [18].

G. Evaluation Of Efficacy Of Screened Isolate For The Crude Oil Degradation By Hydrocarbon Overlay Assay

The isolates were initially screened for biosurfactant production using the hydrocarbon overlay assay, as described by Hanano et al. One microliter of culture was spread on a MSM agar plate coated with 100 µl of kerosene, engine oil, diesel, paraffine, glycerol and incubated for 7 days at 37°C. A colony surrounded by an emulsified halo was considered biosurfactant-producing [17].

H. Antibiotics Susceptibility Profiles of Isolates

Before using this nature isolate, antibiotic susceptibility tests were performed using the Kirby-Bauer disc diffusion method. The clinical and laboratory standard institute, as well as the World Health Organization, recommend this method.

I. Identification of Potential Strain

Identification was done by morphological, growth characteristics and growth pattern on selective media.

J. Extraction of Biosurfactant

For the inoculation of the selected strain, 100 ml of production medium from the pure extracted culture were used and incubated in a rotary shaker at 120 rpm for 7 days at 37°C. The cells were then extracted by centrifugation at 5000rpm for 20 minutes at 4°C to remove cell debris. The pH of the supernatant was adjusted to 2.0 using 1M H_2SO_4. An equal volume of chloroform: methanol in a 2:1 ratio was added. After vigorous shaking, the mixture was left to evaporate overnight. The biosurfactants were discovered in the form of white sediment [19].

K. Dry Weight of Biosurfactant

The sediment was placed in a weighed sterile Petri plate to weigh the dry residue and dried for 25 minutes in a hot air oven at 115°C. The plate was weighted after drying [20]. The dry weight of the biosurfactant was calculated using the following formula:

Dry weight of biosurfactant = (weight of white sediment + dish) - weight of the empty dish

L. Fourier Transform Infrared Spectroscopy (FTIR)

IR Spectrum was used to determine the distinct chemical structure, bonds, and functional groups present in the biosurfactant applying IR spectral analysis. Spectral lines were observed in the vicinity of 4,000- to 400-cm-1 for resolution of spectrum applying FTIR. 5 ml of supernatant were used in this analysis to alloyed with 100 mg of KBr and crush against to obtain a pellet. An infrared absorption frequencies database was used to interpret the peaks [21].

M. Partial Molecular Identification and Phylogenetic tree construction

Taxonomic affiliation of the isolates was determined based on the identity of their 16S rRNA gene sequences with those in the GenBank database and with the nearest type strains in database. To construct a phylogenetic tree, sequences were aligned using Neighbor-Joining method.

N. Applications of lipopeptide biosurfactant

1) *Determination of Antimicrobial Activity:* Antimicrobial activity of selected isolates was determined using partially purified biosurfactant against clinical and environmental bacteria of both Gram-positive and Gram-negative strains including *Salmonella typhi A, Escherichia coli, pseudomonas aeruginosa, Enterobacter aerogenes* by disc diffusion method as described previously [22].
2) *Lipopeptide as Detergent:* Lipopeptide is a possible detergent that lightens and eliminates fabric stains. Lipopeptide's stain-removal ability was tested by putting it to a cloth stained with oil. It was applied, rubbed in thoroughly, and the cloth was allowed to air dry before being removed or lit [23].
3) *Foaming Activity:* Isolated strains were cultivated in 250 mL Erlenmeyer flasks with 100 mL of nutrient broth medium in each. The flasks were incubated for 72 hours at 37°C and 200 rpm in a shaker incubator. Foam activity is measured in the graduated cylinder as the period of foam stability, foam height [24].
4) *Lipopeptide as Plant growth promoting: Pot Assay*

For pot assay, the following treatments were carried out,
1 (Sterile soil + sterile water + seed) control
2 (Sanitized Soil + Lipopeptide + Seed) Lipopeptide Effect
Plant seeds were used for this assay: Phaseolus mungo (Mung), Capsicum annuum (Chilli pepper), Sesamum indicum (Sesame seed) After one week of sowing, the percentage of seed germination in each treated pot was calculated. The root and branch lengths were measured separately for each plant and expressed in cm [25].

III.RESULTS AND DISCUSSION

Microorganisms found in various environmental samples produce a wide range of bioactive compounds. Because of their structural and functional diversity, these BS have piqued the public's interest. Representatives of the genera *Bacillus, Klebsiella*, and *Pseudomonas* are among the bacterial species that commonly produce BS in such environments. To obtain the most potential isolate producing the biosurfactant, an effective screening test should be performed. On this basis, we conducted our study in order to isolate the most promising isolate.

A. Collection of Samples and Isolation of Biosurfactant Producers

Out of six, three samples were collected from Petroleum contaminated soil from different garages of Kataragama, Surat. Marine sample from the respective beaches of Surat. Whey samples were collected from local dairies of Surat. Collected samples (Whey, Marine water and Oil contaminated soil) were streaked, serially diluted and poured on MRS, SCA and BHS agar plates, respectively and studied for their phenotypic characterization.

B. Phenotypic characterization and Biochemical profiling

PS1 isolate was identified as *Klebsiella spp.* based on morphological characterization, growth characterization and biochemical profile. PS2 and M1, M2 as *Bacillus spp.*, and L1 as *Lactobacillus spp.*

C. Primary screening

We used MSM agar, Blood agar, CTAB agar, Tributyrin agar and Modified drop collapse assay for primary screening. In present study, four out of the five isolates (PS1, PS2, M1, M2) grew well on the media. By observing the zone of haemolysis on blood agar, we noted PS1 as β-haemolytic, PS2 and M1 gave α-haemolysis, and M2 and L1 gave γ-haemolysis. CTAB is a dependable, simple, novel, non-tedious method for identifying biosurfactant producers. On CTAB agar, all five isolates (PS1, PS2, M1, M2, L1) shown positive results. Lipase enzyme is produced by biosurfactant producers and can be detected by clearing zones against the turbid background of the plate around isolate colonies. In our study, we looked into all five isolates (PS1, PS2, M1, M2, L1) were discovered to be positive and provided accurate results for the Tributyrin agar plates. In modified drop collapse all five isolates yielded positive results (PS1, PS2, M1, M2, L1- 0.5 cm ,0.2cm, 0.3 cm, 0.1 cm and 0.5 cm diameter respectively).

D. Preliminary characterization

The Biuret assay was used to determine whether or not the isolates were lipopeptide biosurfactant producers. All five isolates (PS1, PS2, M1, M2, L1) were determined to be lipopeptide producers by observing the colour change from light blue to purple, indicating the presence of peptide bonds.

E. Secondary screening

Isolates that yielded appropriate results in primary screening are further studied to determine the actual potentiality and efficacy of the produced biosurfactant using various methods. Oil displacement is a quick and simple confirmatory screening method that measures the diameter of the clear zone of oil on distilled water, which is directly proportional to the biosurfactant potentiality. The zone diameters of the respective isolates are represented in table below. PS1 had the largest diameter of 1.3cm.

The table I also represented the $\%E_{24}$ for other isolates. BATH (Bacterial cell Adherence to Hydrocarbon) test, is a characteristic feature of biosurfactant producing bacteria and is thus known as an indirect screening method. The isolate PS1 had the highest percentage of bacterial adhesion. Lipopeptide biosurfactant contains the fewest sugars. As a result, the isolate with the lowest colour intensity in this assay can be considered the most promising, and the most satisfactory results were obtained with PS1. The emulsified haloes were found against all of the hydrocarbons used, giving the sings of biosurfactant producer bacteria. Because PS1 was identified as the most promising isolate in our study, thus for further study PS1 isolates were considered.

Table I Efficacy of secondary screening methods in predicting BS production.

Isolate No.	ODM (Zone diameter cm)	$\%E_{24}$	%BATH	PSAM
PS1	1.5 cm +++	22% +++	+++	+++
PS2	1.3 cm ++	16% +	+++	++
M1	1.0 cm +	17% ++	++	+
M2	1.4 cm ++	10% +	+	+
L1	1.5 cm+++	N.D. -	+++	+

(Legends: += Positive test, ++=Moderate activity, +++=Very good activity, - =negative, N.D.= Not Determined ODM=Oil Displacement Method, %E24= Emulsification index, BATH= Bacterial Adhesion to Hydrocarbon, PSAM= Phenol Sulphuric Acid Method.)

F. Antibiotic Susceptibility profile of isolate:

PS1 was found to be sensitive to almost all antibiotics used in the disc diffusion assay (Kirby-Bauer Disc Diffusion method). The zone diameter versus the respective antibiotic is shown in the table II and figure 1 below.

Table II Antibiogram susceptibility test

Antibiotics	AS	BA	CF	PC	CH	RC	CL	TE	ZN	GM	AK	GF
Resistogram	IS	S	IS	S	IS	S	IS	IS	R	IS	IS	IS

(Legends: S=Susceptible, IS=Intermediate Susceptible, R=Resistant, AS= Ampicillin/Sulbactam, BA=Co-Trimoxazole, CF=Cefotaxime, PC=Piperacillin,CH=Chloramphenicol, RC=Ciprofloxacin, CL=Ceftizoxime, TE=Tetracycline, ZN=Ofloxacin, GM=Gentamicin, AK=Amikacin, GF=Gatifloxacin.)

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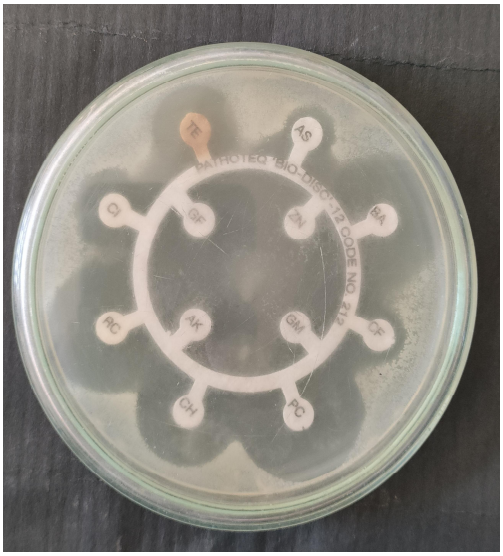


Figure 1 Antibiogram Susceptibility test

G. Extraction of lipopeptide biosurfactant

After 7 days of production and overnight drying of the extracted broth, white coloured precipitates were observed, which were then assumed to have a dry weight of 0.175g/100ml.

H. Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectrum revealed a broad peak at 3500-3000 cm-1, indicated the presence of hydrogen-bonded -OH or -NH functional groups. The presence of carboxyl amide bonds was indicated by a sharp peak at 1636 cm-1. The presence of C-N amide bands was revealed by a small peak at 1267 cm-1, and the presence of C-H hydrocarbon bonds was indicated by a vibrating peak at 1043. A similar range of FT-IR absorption spectra for Lipopeptide biosurfactant was found in the literature. It was done at Ribosome research centre Pvt. Ltd., Near Dhanvantry Pharmacy College, Kim , Surat, Gujarat.

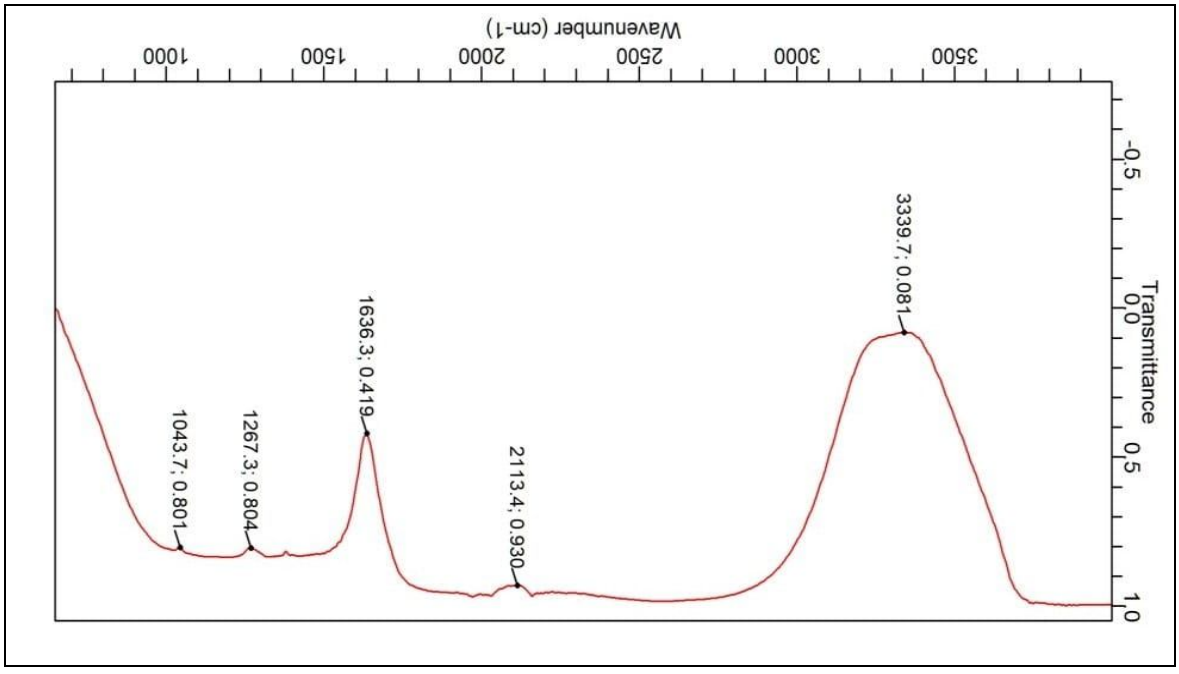


Figure 2 FTIR of extracted biosurfactant produced by PS1

I. Partial Molecular Identifications

By phenotypic characterization, PS1 identified as non-motile gram-negative rod-shaped bacteria. On the selective media CLED and agar plate, it forms irregular, large, round, lactose fermenter mucoid yellowish coloured colonies. By 16s rRNA sequence analysis, the isolate was identified as *Klebsiella quasipneumoniae sub spp, similipneumoniae*, and the sequence has been submitted to NCBI under the accession number ON384439. Phylogenetic tree depicting the evolutionary relationship of *Klebsiella quasipneumoniae subsp. silmilipneumoniae* with selected *Klebsiella spp.* Several authors have investigated, reviewed, and proposed the use of biosurfactant isolates from various genera in the field of bio-remediation and enhanced oil recovery, and *Klebsiella spp.* is one of the most novel spp that has been reported in this category. It was done at Gene Xplore Diagnostics & Research centre Pvt. Ltd., Ahmedabad, Gujarat.

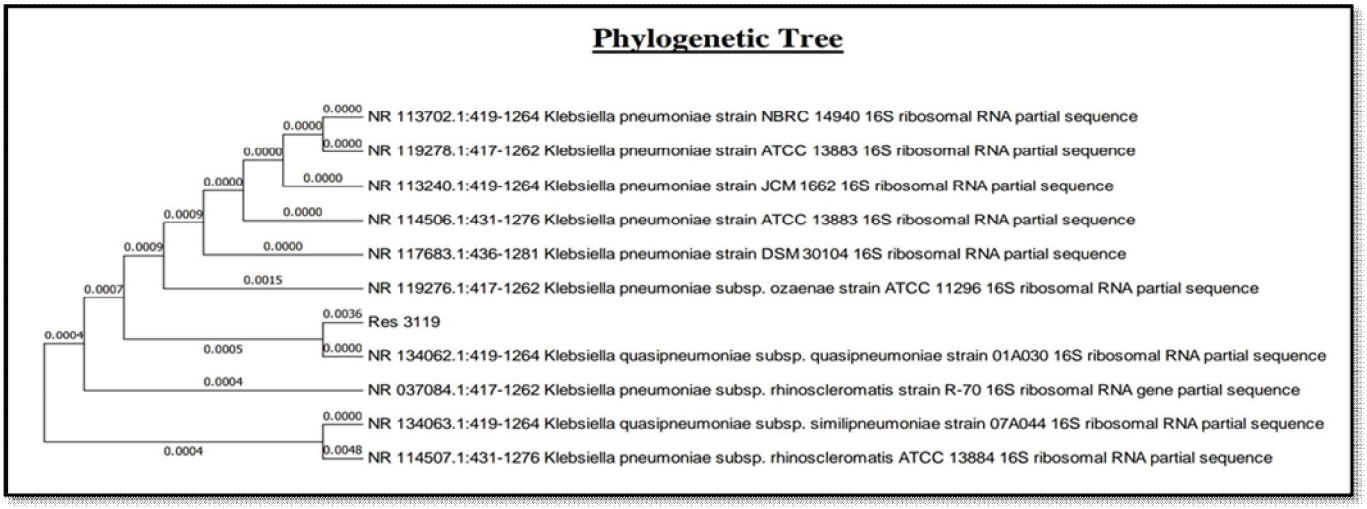


Figure 3 The neighbour-joining tree based on the 16S rRNA sequence, demonstrating the phylogenetic position of strain PS1

J. Applications

1) *Antimicrobial Activity:* The antimicrobial activity of crude lipopeptide biosurfactant was determined by measuring the zone of inhibition against the test organisms, *Salmonella typhi*, *Escherichia coli*, *Enterobacter aerogenes*. *Pseudomonas aeruginosa* and *Styaphylococcus aureus.* Among all, only *Pseudomonas aeruginosa* show resistant to crude lipopeptide biosurfactant.
2) *Detergent:* Lipopeptide, as a biosurfactant, may have detergent activity, and was tested by applying it to a cloth with oil stains and then rubbing crude surfactant on it; we observed the removal of oil stain of successfully within 5 minutes
3) *Foaming Agent:* Lipopeptides can be used in the detergent industry by its foaming activity. Foam production capabilities was checked by mixing 2 ml of biosurfactant in 10 ml of sterile nutrient broth and by incubating in a rotary shaker for 72 hours. We noted a well-developed foam layer with a height of 1.0±0.2 cm.
4) *Pot Assay:* Lipopeptides have been shown to be effective plant growth promoters, which was tested by performing a pot assay. We sow mung seeds in two pots, one with and one without Lipopeptide biosurfactant. After 20 days, the height of the root, stems, and number of stems were measured, and the results of the same are shown in the table V.

Table III Germination and Mean Root and Shoot Growth

Sample	No. of germinated seeds out of 10	Mean root growth (cm)	Mean shoot growth (cm)
Phaseolus mungo (Mung)	8	8.2	11.74
Sesamum indicum (Sesame seed)	9	5.4	10.45
Capsicum annuum (Chilli paper)	7	7.1	11.56

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IV. CONCLUSIONS

Due to environmental factors, the need for a chemical surfactant alternative is becoming more pressing in the modern era. Lipopeptide biosurfactants are one such societal alternative that is produced by a variety of microorganisms and families. Lipopeptides are environmentally friendly biosurfactants that can be used in a variety of fields including oil recovery, medicine, cosmetics, agriculture, and others.

Six biosurfactant producing organisms were obtained and identified using phenotypic characteristics in the current study. Primary screening for haemolytic activity, MSM plate, Tributyrin plate, CTAB plate, and drop collapse test were done for all isolates to determine their biosurfactant production capabilities. Five of the six produced positive results and were used further for production potential check. Secondary screening for the potentiality of the produced microbial biosurfactant was carried out by the Oil displacement assay, Emulsification index, BATH assay, and Phenol Sulphuric Acid assay. Among the five isolates we received, isolate PS1 yielded the most promising results.

PS1 biosurfactant was extracted, and white biosurfactant precipitates were observed. FT-IR analysis was used to characterise the biosurfactant, which confirmed the production of Lipopeptide biosurfactant. 16s rRNA sequencing results revealed PS1 as *Klebsiella quasipneumoniae subsp. Silmilipneumoniae* (NCBI accession number: ON384439). Lipopeptide was primarily studied for antibacterial, foaming activities, potential detergent and plant growth promoting properties, and potential applications.

V. ACKNOWLEDGMENT

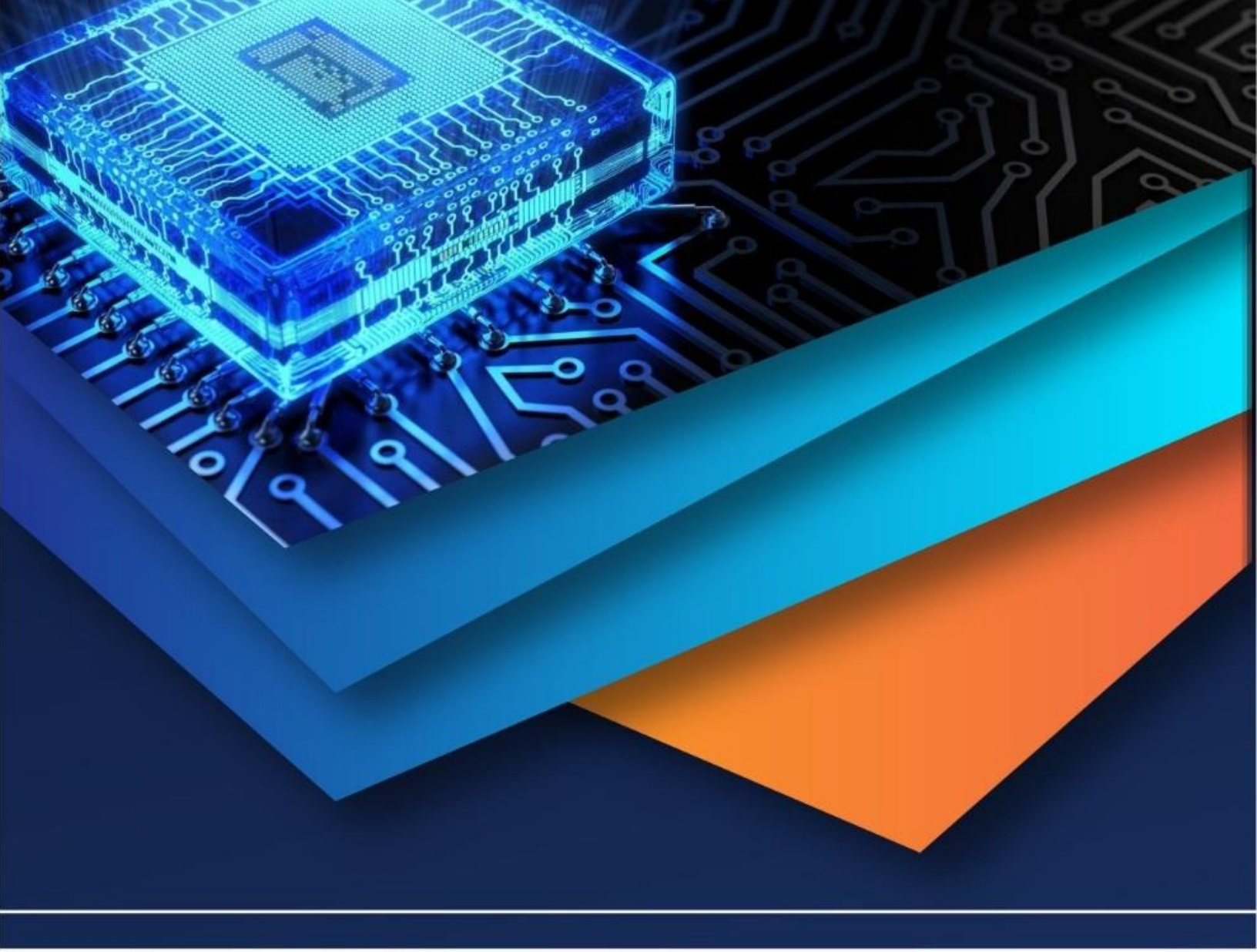
All researchers are sincerely acknowledged and thanked for their significant contributions to this endeavor.

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