International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Isolation and Characterization of Biosurfactant from *Klebsiella pneumoniae subsp.rhinoscleromatis*

Ashwini Thorat[1], Vedanti Dikey[1], Sakshi Deshpande[1], Rohini Pungle[2]*

[1, 2]Department of Biotechnology and Bioinformatics, Deogiri College, Chhatrapati Sambhajinagar, Maharashtra, India

*Corresponding Author: Dr. Rohini Pungle,
Department of Biotechnology and Bioinformatics,
Deogiri College, Chhatrapati Sambhajinagar,
Maharashtra, India
E-mail: patilrohini194@gmail.com
Mobile No.: +91 9403888372

Abstract: ***Biosurfactant is surface active compounds produced by microorganisms. Oil contaminated soil sample were collected from motor garage and microorganisms were screened for biosurfactant production using enrichment culture techniques. Various screening methods, including emulsification index, drop collapse method, CTAB agar method, were applied to identify the most efficient biosurfactant producer. S4.1 was found to be maximum producer of biosurfactant which has been identified as Klebsiella Pneumoniae subsp.rhinoscleromatis by 16S rRNA sequencing. Biosurfactant production by S4.1was optimum at incubation period of 96 hr., pH 5 and 1% glucose as carbon source. Cell free extract consisting biosurfactant has maximum activity at acidic and alkaline conditions and at temperature 30°C. The oil spreading method and hemolytic activity confirmed presence of biosurfactant in extracted and purified biosurfactant. The thin layer chromatography (TLC) and fourier transform infrared spectroscopy (FTIR) confirmed the purified biosurfactant as glycolipoprotein. Biosurfactant was found to have antimicrobial activity against E.coli and Pseudomonas spp. and had ability to form micelle.***

Keywords: Biosurfactant, emulsification index, drop collapse method, CTAB agar, *Klebsiella pneumoniae subsp.rhinoscleromatis*

1. Introduction

Surfactants are able to reduce tension at phase interfaces (Soumik Banerjee et al 2023). It can also emulsify oil in water and water in oil as well as help in stable gel and foam formation. The biosurfactant are classified as synthetic or microbial (biosurfactant) according to origin (Thiago R Bjerk, 2021). Synthetic or chemical surfactants are used at high level still they may have an impact on the environment and human health. Wide application of synthetic surfactants that are man-made organic molecules in industries producing soaps, detergents, shampoos, cosmetics, toothpaste, and pharmaceuticals. High manufacturing and energy expenses, low solubility, surface charge, diversity, and other physicochemical characteristics limited their use in industries.

Hence, present day need is to develop alternative molecules such as biosurfactants. Biosurfactants are the molecules produced by the microorganisms. As biosurfactant has biological origin, it is biodegradable and environmentally safe. Biosurfactant are superior than synthetic one in having easy degradation by microbes, low toxicity, good compatibility and digestibility with other living organisms, can be produced from cheap raw materials that are easily available in large quantities i.e. use of the waste material. It also exhibits emulsification capacity (Nitin Deshmukh et al, 2023).

Biosurfactants are amphiphilic molecules consisting hydrophobic tail and hydrophilic head moieties. They interact with surfaces of diverse polarities and reduce the surface and interfacial tension of solutions. Biosurfactants can either be secreted as extracellular molecules by microorganisms or be associated directly with the microbial cell surface itself. (Soumik Banerjee, et al, 2023). Biosurfactant are active compounds that are produced at the microbial cell surface or excreted and reduce surface and interfacial tension (Wuyang Sun et al, 2018).

Biosurfactants are molecules that disperse hydrocarbons into small droplets, reducing their surface tension and increasing their availability for microorganisms. Surfactants are classified into four types based on the charge of their hydrophilic head:

The mainly biosurfactants are categorised by their chemical structure and microbial origin, and include glycolipids, lipopeptides, phospholipids, Polymeric biosurfactants, Particulate biosurfactants, Fatty acids. Biosurfactant has properties like reduces the surface tension of water, excellent capacity of forming micelle, lethality of biosurfactants is very low, have digestibility (Nikhil Shah et al, 2016).

These biosurfactants have importance in various industries such as in chemical, cosmetic, pharmaceutical, agricultural and food. Its impact is emphasized when it governs a property of emulsification. Microbes by having emulsification activity degrades the pollutants by enhancing the pseudo solubilization. The use of biosurfactants is offered as a best alternate option because of its versatility, biodegradability and ecofriendly nature that maintains sustainability of the environment in a much-desired way (Wuyang Sun et al, 2018).

Above a critical concentration, surfactant monomers self-aggregate into supramolecular structures called micelle. Micelle contains a hydrophobic core and a charged or hydrophilic surface. The core of micelle solubilizes hydrophobic substances like oils or poorly soluble drugs and

allow them to be dispersed in aqueous solution (Mayer et al, 2024).

The present work deals with objectives to isolate and identify an efficient biosurfactant-producing bacterial culture with optimizing the production condition for biosurfactant. Antimicrobial and micelle formation potential of purified biosurfactant.

2. Materials and Methods

Material
All materials were purchased from S. D. fine chemicals and Hi Media. Minimal broth Davis w/o Dextrose, Agar agar powder (Bacteriological), Glucose Powder, Oil (used motar oil), CTAB, Methylene blue, Saline, Phosphate, Buffer, Crystal violet, Gram's iodine, Ethanol, Safranin / Carbol fuchsin. The instrumentation Autoclave, Centrifuge, Laminar air flow, Vortex mixer, Micro oven, Hot air oven etc.

Methods:
1) Sample collection:
1 gm of four soil samples was collected from the oil contaminated soil which was placed at motar garage, Rachanakar colony, New Osmanpura, Chhatrapati Sambhaji Nagar in sterile eppendrof tube.

2) Isolation, purification, preservation of bacteria:
For isolation of biosurfactant producer enrichment method was utilized. For enrichment of biosurfactant producing bacteria minimal media with 1% glucose and 10% oil used.

1gm of soil sample was mixed properly in 10ml sterile saline by vigorously shaking. 1 ml of sample was added in 10 ml of minimal broth with 1% glucose and 1 ml oil (10%). it was incubated for 3 day at 37°C.

The inoculum was streaked on minimal agar plate with 1% glucose and 10% oil. The plates were incubated at 37°c for 3 days. The obtained mix culture was purified by restreaking. The obtained bacterial culture were preserved on slant of minimal agar with 1% glucose and 10% oil at 4°C.

3. Screening for bio surfactant producer:

3.1 Drop collapse method (DCM)

A single colony of each bacteria was inoculated in 5ml minimal broth and incubated at 30°C for 96hr. The culture broth was centrifuged at 8000 rpm for 20 min to obtain cell-free supernatant (CFS). The 50µl supernatant was added to the 1 ml of surface of oil. The shape of drop on the oil surface was observed after 1 min. DCM was carried out for evaluation of the biosurfactant activity. Briefly if the droplets are in a flat shape when the drops drip onto the seal film, the reactions are positive. But if the droplets are in a spherical shape, the reactions are negative (Wuyang Sun et al, 2018).

3.2 CTAB Agar method

A single colony of each bacteria was inoculated in 5ml minimal broth and incubated at 30°C for 96hr. The culture broth was centrifuged at 8000 rpm for 20 min to obtain cell-free supernatant

CTAB agar plates were prepared by adding 0.04 g of Cetyltrimethyl ammonium bromide 0.001 g Methylene blue and 3 g of agar agar to 200 ml of distilled water, adjusted to pH 7 and sterilized. Hole (6 mm diameter) was made in the CTAB plate, and approximately 100 µL of cell-free supernatant was loaded inside each hole. Plates were incubated at 30^0C for 96hr. Cell free supernatant containing anionic surfactant produced blue halos around the wells in which they were place (Rani M et al, 220).

The cultures were spot inoculated on CTAB agar plates. Plates were incubated at 30^0C for 96 hr. The blue colour around the colony shows biosurfactant production.

3.3 Emulsification method

The emulsification index (EL) is used to characterize the emulsifying activity of the biosurfactant. 2ml of cell free suspension (CFS) and 2ml of crude oil vortexed vigorously for 5 min. It was placed vertically at room temperature without disturbance for 96hr. The EL is calculated as follow (Haytham M.M. Ibrahim, 2016).

EL = (height of the emulsion of layer/total height of liquid)×100

4. Molecular Identification

The isolated bacterial culture 4.1 was identified by 16S rRNA sequencing at National Collection of Industrial Microorganisms, Pune (Nwaguma, I.V. et al 2016, Kaur H. et al 2022).

5. Factor affecting production of biosurfactant

5.1. Incubation period

To find out optimum incubation period single colony was inoculated 5mlof minimal media with oil. The cultures were incubated at different hrs (24hr, 48hr, 72hr, 96hr, 120hr, 144hr). After completing incubation the inoculum was centrifuged at 8000rpm for 20min. The cell free supernatant was tested for emulsification. The emulsifation indexes were measured (Fardami A.Y et al, 2022).

5.2. pH

The minimal broth was prepared and adjusted the pH and sterilized it, single colony was inoculated in each test tube and broth was incubated at 96hrs. The cell free culture was centrifuged at 8000 rpm for 20 min. The emulsifying indexes were measured after 24hrs (P.Anna Joice and R. Parthasarathi, 2014).

5.3. Carbon source

The carbon source is one of important parameter to affecting production of biosrfactant. Accordingly, the minimal broth was prepared at different concentration of carbon source. The concentration of glucose was varied as 0.5%, 1%, 1.5%. A

colony was inoculated in a broth incubate for 96hr. To obtain cell free culture, inoculum was centrifuged at 8000 rpm for 20 min. The emulsifying indexes were measured (Fardami A.Y et al, 2022).

5.4. Factor affecting activity of Biosurfactant:

5.4.1 Effect of pH:

The activity of biosurfactant is affected by pH. The stability of biosurfactant was found out by taking **c**ell free supernatant consisting of biosurfactant by adjusting to various pH values from (pH 3, pH5, pH6, pH8, pH9, pH10, pH12, pH14) with 1N HCL or 1N NaOH. The emulsifying indexes were measured for each pH. [13]

5.4.2 Effect of Temperature:

The maximum activity of biosurfactant was analysed by maintaining the cell free supernatant at a constant temperature in the range (30°C-80°C) for 30 min. And then cooled to room temperature, before measuring the emulsification activity (Foukia E. Mouafia et at, 2014).

6. Production, Extraction and Purification

6.1 Production of biosurfactant:

A single colony of S4.1 was inoculated in 5ml minimal broth and incubated at 37°C for 96hr. the 5ml culture broth transfer into 100ml minimal broth (production media) was incubated in shaking incubator at 121 rpm. For 168 hr.

Biosurfactant extraction was performed as described by. The 96hr old culture centrifuged and collect Supernatants. Supernatants were acidified to pH 2 with 1 M concentrated HCl and stored at 4°C for 24hr. It was centrifuged at 8000 rpm for 20 min and the precipitate was recovered. The precipitate was then neutralized with 0.067 M phosphate buffer at pH 7. After placing this solution in a falcon tube an equal volume of ethyl acetate was added. After vigorously shaking for 10 min and decantation for 1 hr, the organic phase was set aside and the process was repeated several times. The organic phase was recovered and pooled with the first recovered solution. Ethyl acetate was then evaporated. and the active fractions were stored at 20°C (Chotard et al, 2010) [14].

Confirmation of purified Biosurfactant was carried out by drop collapse method, Oil spread method, haemolytic activity.

6.2 Drop collapse Method:

The 50µl supernatant was added to the 1 ml of surface of oil. The shape of drop on the oil surface was observed after 1 min.

6.3 Oil spreading method:

In the oil spreading technique 50 mL of distilled water was added to the Petri plate followed by addition of 100 µL of crude oil to the surface of the water. Then, 10 µL of purified biosurfactant was dropped on the crude oil surface. The diameter of the clear zone on the oil surface was measured and compared with distilled water as a negative control (Foukia E. Mouafia et al, 2016).

6.4 Hemolytic activity:

In Hemolytic activity sterile nutrient agar medium was cooled to 45 °C, and then 5% (v/v) of fresh blood was added, mixed gently, and poured into sterile Petri dishes. The culture supernatants was transferred into 6mm well punctured in the blood agar plates. The plates were incubated at 37 °C for 24–48 hr. The development of clear zone (hemolysis) around the wells indicates the possibility of biosurfactant production (Haytham M.M. Ibrahim, 2016).

7. Charaterization of the biosurfactant

7.1 Thin layer chromatography

The biosurfactant was subjected for thin layer chromatography (TLC) for analysis he biosurfactant. Solvent systeme used for TLC comprises chloroform–methanol-water-acetic acid in the ratio 65:25:2:1.5. The biosurfactant obtained as a fermentation product was dissolved in methanol. To identify the components of biosurfactant (proteins, carbohydrates and lipids) three TLC plates were run and applied locating reagents ninhydrin reagent, sulfuric acid and iodine vapours (Soumik Banerjee et al, 2023).

7.2 Fourier transform infra-red spectroscopy (FTIR)

For the confirmation of biosurfactant the FTIR analysis was performed at Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar.

8. Applications

8.1 Micelle Formation:

To form the micelle the solution of saffranine was made in distilled water (0.0001gm in100ml). The biosurfactant at different concentration (5 ul, 10 ul, 15 ul, 20 ul) was added in 1ml of saffranine solution and vortex it for 10min. The absorbance of sample was measured at 520nm. The stability of micelle was checked for 24hr. The vortexed mixture was kept undisturbed for 24hr and absorbance was measured. The experiment was performed in triplicate.

8.2 Antimicrobial Activity

Antibacterial activity of partially purified Biosurfactant using agar diffusion method was performed. Firstly, the nutrient agar medium was prepared (basal agar and soft agar) 15 ml basal nutrient agar medium were poured in Petri plate and allow to solidify for few minutes. The soft nutrient agar was added on basal agar with 100ul of *E.coli* and *Pseudomonas aeruginosa* culture. The 6mm wells was made on the agar plate and 10ul of the partially purified biosurfactant was added in first well and chloroform: methanol (2:1) was added in second well as a control. The plate was incubated at 4°C for 10 min in refrigerator. After that plates were incubated at 30°C for 96 hours. The presence

of clear zone marked the antibacterial activity of biosurfactant (Foukia E. Mouafia et al, 2022).

9. Result and Discussion

1) Sample collection and isolation purification of biosurfactant producer:

Total 11 culture were isolated. All were gram positive in nature. Some samples were cocci and short rod.

2) Screening for biosurfactant producer:

a) Drop Collapse method (DCM):

In the drop collapse method, water was used as negative control. The isolates were observed for positive result as sample drop was collapse on oil. The S1*, S4.2, S4*, S4.1. had showed the significant result (Table No. 1, Fig No. 1)

Table 1: Drop Collapse method

Sample	DCM
Water	0.5
Sample 1*	0.55
Sample 2	0.5
Sample 2.1	0.55
Sample 3.2	0.3
Sample 4	0.25
Sample 4*	0.65
Sample 4.1	0.3
Sample4.2	0.6
Sample3*	0.6
Sample 2*	0.5

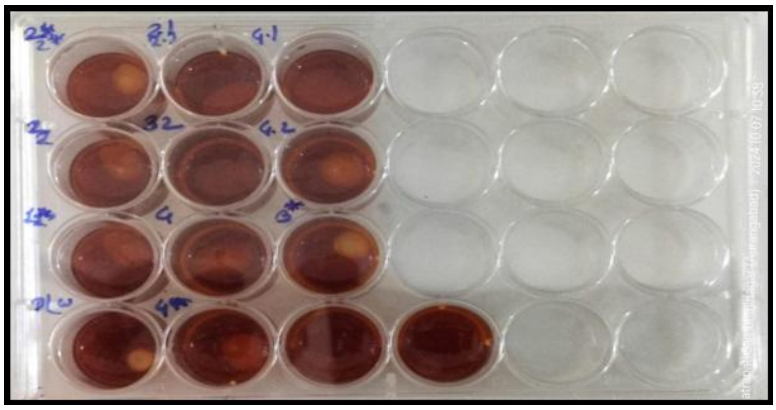


Figure 1: Drop Collapse method

3) CTAB method

In the CTAB method when the supernatant was added into the well and incubated it was observed the blue holes (Fig No.2). Indicating the production of biosurfactant by bacterial culture.

When the colonies were spot inoculated on CTAB media. The blue holes were observed around the colonies and also colonies turned blue colour. It confirms the production of biosurfactants. Some samples are show blue colonies that is (S4.1, S4*, S4, S1*, S3*, S3.2). Some samples are not show blue colonies that is (S1, S4.2, S4*, S2.1) (Fig.No.3).

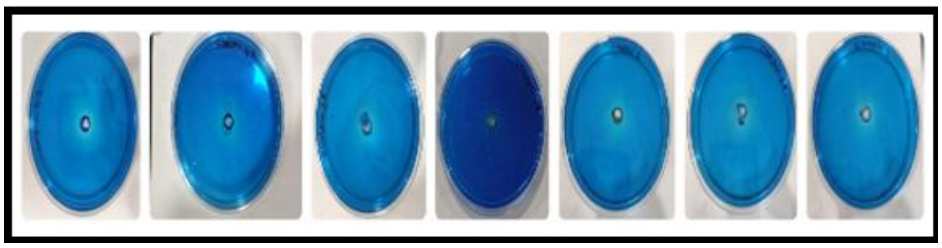


Figure 2: CTAB Method

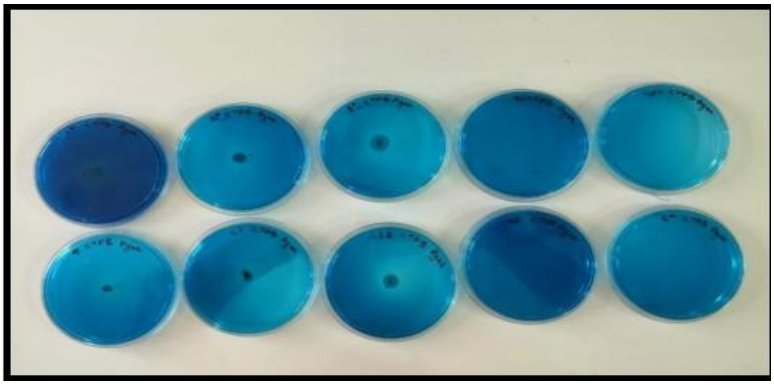


Figure 3: CTAB Method: Spot inoculation

4) Emulsification method

For emulsification index tween 80 was positive control. By comparing the emulsification index, it was found that S4.1 was good emulsifier as well as biosurfactant (Table No. 2). The S4.1 was consistently producing same level of biosurfactant. Thus, for further studies we have selected sample S4.1.

Table 2: Emulsification index (EL)

Samples	Emulsification Index (EI)
Sample 4.1	71.85
Sample 2	92.00
Sample 4	97.5
Sample 1*	120.1
Sample 4*	102
Sample 2*	132.7
Sample 3*	92.82
Tween 80	125

5) Molecular Identification:

Isolated bacterial culture sample 4.1 was identified at National Collection of Industrial Microorganisms (NCIM) by 16S rRNA sequencing (600bp). Extracted Genomic DNA from the bacterial isolate was used as a template for PCR amplification of the 16S rRNA gene using universal primers 27F and 1492R. The purified PCR product was sequenced. The obtained sequence was analyzed using the BLAST tool in the NCBI GenBank database. The isolate showed 100% sequence similarity with *Klebsiella Pneumoniae subsp.rhinoscleromatis* (Accession No. NR_037084.1).

Hence, the bacterial isolate was identified as *Klebsiella Pneumoniae subsp.rhinoscleromatis* based on 16S rRNA gene sequence analysis. *Klebsiella Pneumoniae spp.* were isolated from the hydrocarbon contaminated soil and able to produce the biosurfactant was found by (Nwaguma, I.V. et al 2016, Kaur, H. et al, 2022).

6) Factor affecting production of biosurfactant

According to Fig No.4, optimum condition for maximum biosurfactant productivity by culture was 96hr which was in accordance to according (Fardami A.Y et al, 2022). The optimum condition of pH observed for higher production of biosurfactant at pH 5.0 which was lower than observed (Fardami A.Y et al, 2022) (Fig.No.5). The optimum carbon source for higher production of biosurfactant was found to be 1% of glucose. (Fig No.6)

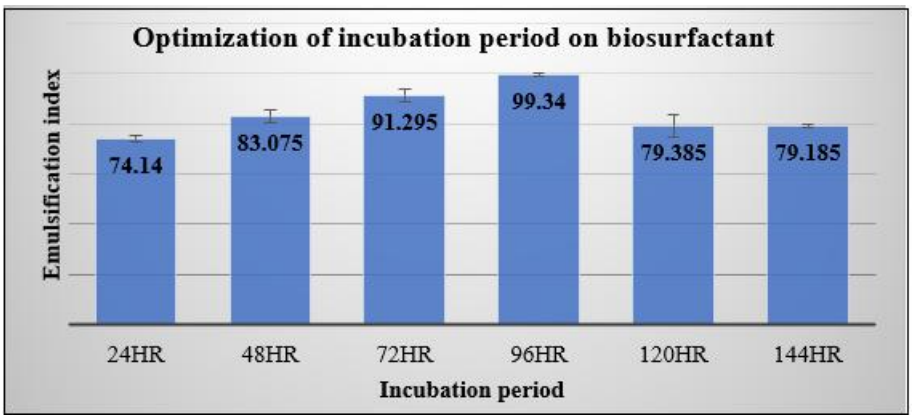


Figure 4: Optimization of Incubation period on biosurfactant

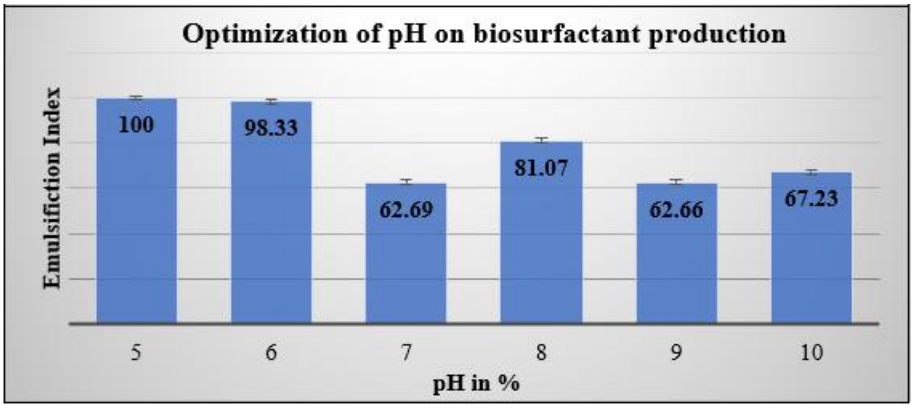


Figure 5: Optimization of pH on biosurfactant production

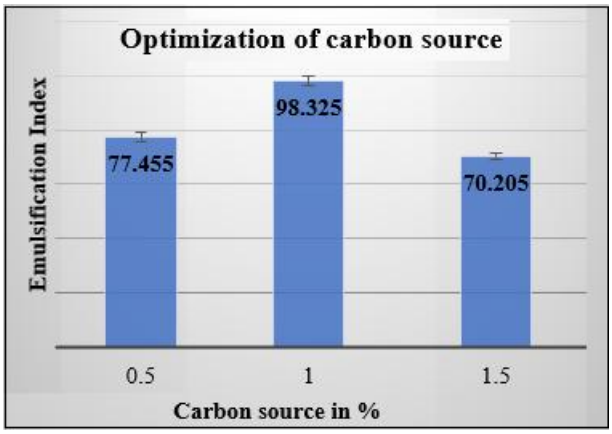


Figure 6: Effect of Carbon source

7) Factor affecting activity of biosurfactant

Stability of biosurfactant was observed between the pH 3, 5, 6, 8, 9, 10, 12, 14. The biosurfactant was stable at acidic and alkaline condition. The optimum pH for the activity was pH5.0 but also have good activity at alkaline pH. Subsequent increase in pH level showed lower activity by emulsification index (Fig. No.7). Temperature is one of the critical parameter that greatly affect biosurfactant activity. The optimum activity was found at 30°C temperature (Fig. No.8). The results contradict (Foukia E. Mouafia et al, 2022) where findings were pH7 and temperature 50^0C.

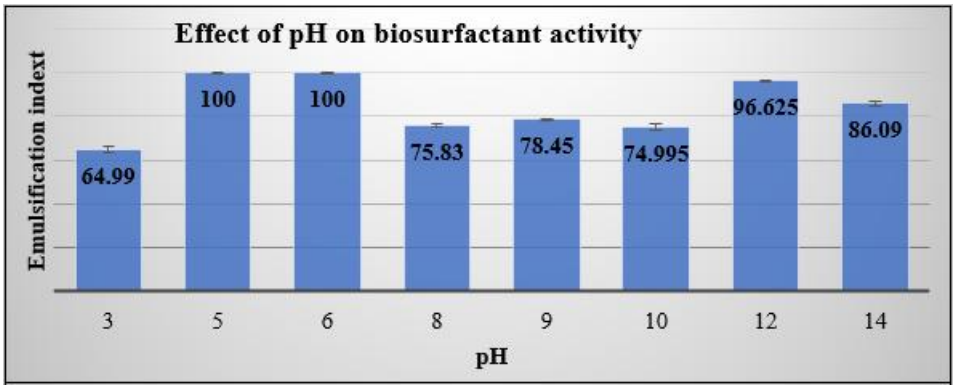


Figure 7: Effect of pH on biosurfactant activity

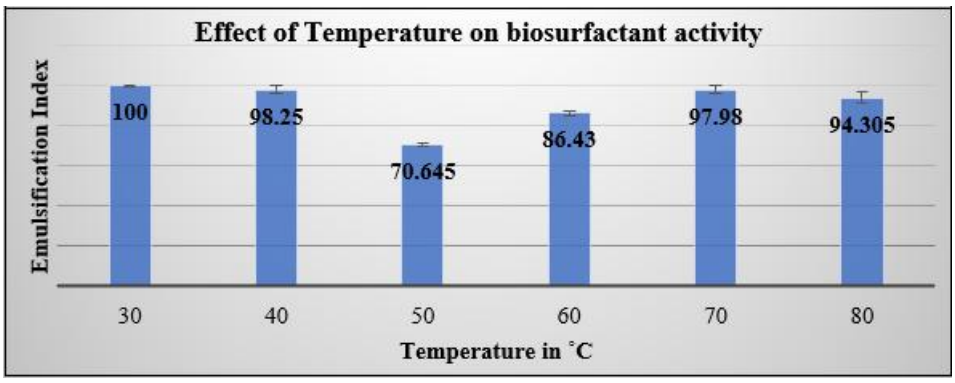


Figure 8: Effect of temperature on biosurfactant activity

8) Production, Extraction and Purification of biosurfactant:

The production of biosurfactant was carried out at optimum conditions for growth of S4.1 in batch culture (Fig. No.10). With solvent extraction method which utilized the solvent ethyl acetate.

Figure 10: Production media

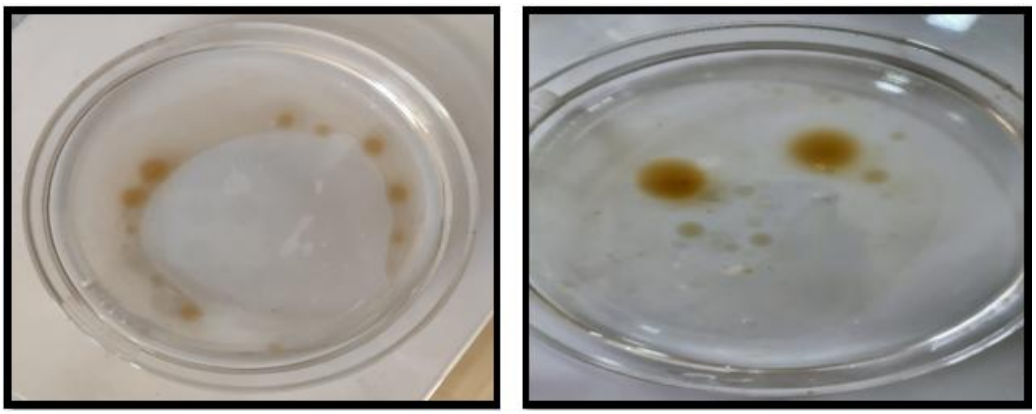
9) Drop collapse method

Fig. No. 11 was observed for drop collapse by 1cm confirming extracted biosurfactant which was effective than tween 80 which had drop collapse of 0.5cm. The negative control had drop collab of 0.3cm.

Figure 11: Drop collab for extracted Biosurfactant

10) Oil spreading

If extracted material is really acting like a biosurfactant, the oil is displaced and clearing zone is formed. The diameter of this clearing zone on the oil surface correlates to surfactant activity, also called oil displacement activity. From the Fig. No. 12 it is clear that extracted product was biosurfactant as it had clearing zone of 3cm. Similar types of clear zones were observed (Foukia E. Mouafia et al, 2022).



Test Control

Figure 12: Oil spreading

11) Haemolytic activity

The extracted biosurfactant was used for haemolytic activity. From Fig. No.13 the zone of clearance was observed as 1.5cm. Thus, it could be accomplished that extracted product was biosurfactant as with the work done (Foukia E. Mouafia et al, 2022).

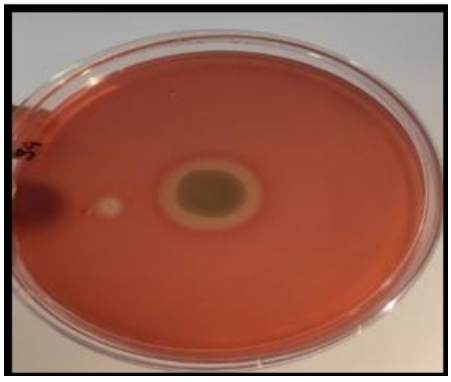


Figure 13: Haemolytic activity

10. Characterization of biosurfactant

10.1 Thin layer chromatography

After extraction surface active compound produced the sample where analyzed by TLC which is the simple, fast and low-cost method. The combination of the silica as the stationary phase and chloroform: methanol: distilled water: acetic acid (65:25:2:1.5) as the mobile phase enabled compound separation. The ninhydrin shows that amino acid where present in the sample the RF value is (0.42, 0.92) identified lipopeptide which show red and pinkish spots. Sulfuric acid show that glycolipid present and which produced brown spot. Its RF value was (0.76). Iodin vapours show that lipids were present which produced the yellow spots. The RF values were (0.62, 0.06) (Table No. 3). The parallel outcomes were obtained (Chotard et al, 2022). From the table No. 9 it can be concluded that the biosurfactant had glycolipoprotein nature which was confirmed by FTIR.

Table 3: Thin layer chromatography

Sr. No.	Locating reagent	R.F. values	Identified compound
1.	Ninhydrin	0.42, 0.92	Peptide
2.	Sulfuric acid	0.76	Carbohydrate
3.	Iodine vapours	0.62, 0.06	Lipids

10.2 Fourier transform infra-red spectroscopy (FTIR)

The FTIR spectrum (Fig No.14, Table No. 4) of column purified biosurfactant revealed important bands at 3344 cm−1, 2921 cm−1, 2850 cm−1, 1742 cm−1, 1728 cm−1, 1655 cm −1, 1452 cm−1, 1376 cm−1, 1250 cm−1, 1218 cm−1, 1113 cm−1 and 1022 cm−1. For interpretation of various functional groups present in the biosurfactant. Due to the presence of hydrogen bonding, the appearance of a strong and broad band of the hydroxyl group (-OH) free stretch was observed at 3344 cm−1 (Brinda C. M et al, 2023). The occurrence of C-H stretching vibrations of hydrocarbon chain of alkyl (CH2-CH3) groups was confirmed by the absorption band observed at 2921 cm−1(Patowary K et al, 2024). Carbonyl group showing peaks at 1742 cm−1 (Huria Rizvi et al, 2024). Characteristic carbonyl stretching band which denotes the presence of ester compounds was found at 1728 cm−1 (Patowary K et al, 2024). The stretching of COO − group was asserted through the deformation vibration at 1655 cm−1 (Walter Chinaka John et al, 2021). Hydroxyl group showing peaks at 1452 cm−1 (Badiaa Essghaier et al, 2023). Glycolipid showing peaks at 1376 cm−1 (Mayer et al, 224). Aliphatic group showing peaks at 1250 cm−1[19]. The absorption band found at 1218 cm−1[13], 1022 cm−1(Patowary K et al, 2024) was the characteristics of the glycosidic bond (C-O-C) present in the molecule.

Thus, FTIR analysis confirms the biosurfactant was a glycolipopeptide in nature. produced *Klebsiella Pneumoniae subsp.rhinoscleromatis*. The results were similar obtained by (Kaur, H. et al, 2022, Ebtehag A.E et al, 2021).

Table 4: FTIR

Sr. No.	Peaks	Functional groups
1	3344.12	Hydroxyl group
2	2921.75	Alkyl chain
3	2850.92	Aliphatic
4	1742.85	Carbonyl
5	1728.33	Esters
6	1655.09	Carbonyl
7	1452.89	Peptides
8	1376.91	Glycolipid
9	1250.61	Aliphatic
10	1218.15	Glycosidic bond
11	1113.35	Aliphatic ether
12	1022.46	Glycosidic

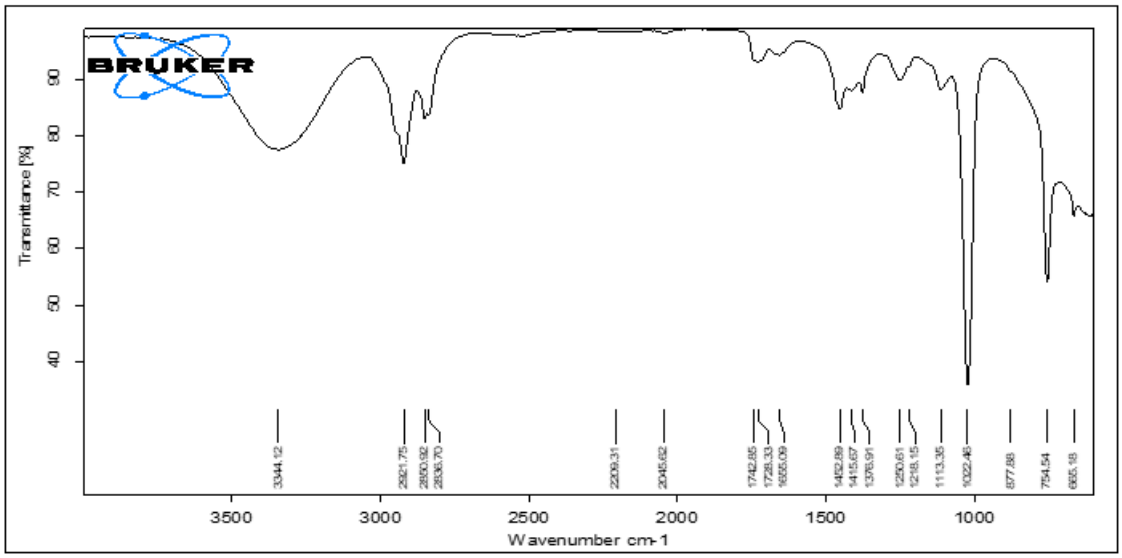


Figure 14: FTIR analysis

11. Application

11.1 Micelle Formation:

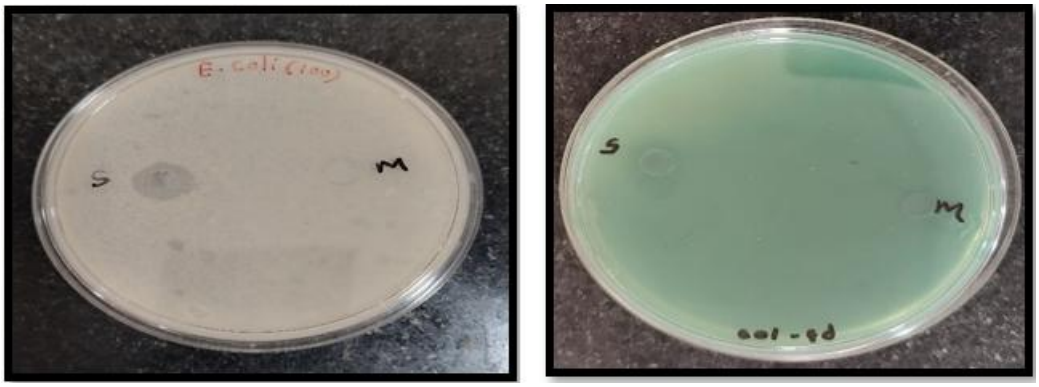
At 0hr 15µl of biosurfactant was found to be optimum as recurring results. The same result was found after 24hr. Thus, 15µl of biosurfactant was efficiently forming the stable micelle (Table No.5).

Table 5: Micelle Formation

Sr. No.	Volume of biosurfactant (µl)	Absorbance at 520nm	
		0hr	24hr
1.	10	0.02 ±0.016	0.04 ±0.012
2.	15	0.04	0.02 ±0.016
3.	20	0.04±0.0188	0.04 ±0.009
4.	Control	0.08 ±0.004	0.08 ±0.004

11.2 Antimicrobial Activity

The biosurfactants have the antimicrobial activity. Antimicrobial assay of biosurfactants was carried out using well diffusion method against bacteria such as *E coli, Pseudomonas spp.* The extracted biosurfactant showed antimicrobial activity against *E coli, Pseudomonas spp*. (Fig.No.15) The biosurfactant had efficient antimicrobial activity on *E coli (13mm)* than *Pseudomonas spp*. (08mm). Methanol: chloroform (2:1) used as a negative control was observed for no zone of inhibition. Thus, isolated biosurfactant have potential to inhibit the growth of Gram-negative organisms.



E. Coli Pseudomonas

Figure 15: Antimicrobial activity

12. Conclusion

11 bacterial isolates were isolated from oil contaminated soil sample. S4.1 (*Klebsiella Pneumoniae subsp.rhinoscleromatis*) was best producer for biosurfactant which was screened out by experimental methods, emulsification, oil spreading, drop collapse, CTAB (cetyltrimethylammonium bromide) agar plate method. Biosurfactant produced was showing maximum activity at acidic and alkaline conditions at temperature 30^0C. Production of biosurfactant was optimum at incubation period 96hr., pH5, and 1% carbon source. TLC analysis pointed out that the biosurfactant produced was of glycolipoprotein in nature. FTIR spectroscopy analysis showed the glycolipid, ester, aliphatic and hydroxyl/carboxyl glycosidic peaks supporting the findings obtained by TLC. These complementry physico- chemical and bioassay convulsively demonstrate that isolated biosurfactant are active biosurfactant. Biosurfactant has both antimicrobial activity ability to form stable micelle. It can be concluded that S4.1 is the best biosurfactant producer.

References

[1] Soumik Banerjee, Uma Ghosh, Production, purification and characterization of biosurfactant isolated from Bacillus oceanisediminis H2. Fernandes, N.d.A.T.; Simões, L.A. Dias, D.R. Biosurfactants Produced by Yeasts. Fermentation, Screening, Recovery, Purification, Characterization, and Applications. Fermentation 2023, 9, 207. https://doi.org/10.3390/ 22 February 2023.

[2] Thiago R Bjerk, Patricia Severino, Sona Jain, Conrado Marques, Amelia M Silva, Tatiana Pashirova, Eliana B Souto. Biosurfactants: Properties and Applications in Drug Delivery, Biotechnology and Ecotoxicology. 2021 Aug 13;8(8):115. Doi: 10.3390/bioengineering8080115.

[3] Nitin Deshmukh, Gunderao Kathwate. Biosurfactant Production by Pseudomonas aeruginosa Strain LTR1 and its Application. Volume 13, Issue 1, 2023, 10. https://doi.org/10.33263/BRIAC131.010.

[4] Nikhil Shah, Rohit Nikam, Shikha Gaikwad3, Vaijayanti Sapre4, Jaspal Kaur. Biosurfactant: Types, Detection Methods, Importance and Applications. 10.5958/2394-5478.2016.00002.9.

[5] Wuyang Sun, ab Wenrui Cao, c Mingyu Jiang, c Gaowa Saren, c Jiwei Liu, abd Jiangfei Cao, Imran Ali, ab Xinke Yu, Changsheng Peng and Iffat Naz, Isolation and characterization of biosurfactant producing and diesel oil degrading Pseudomonas sp. CQ2 from Changqing oil field, China, RSC Adv., 2018, 8, 3971

[6] Mayer, J.D, Rauscher, R.M, Fritz, S.R Fang Y, Billiot, E.J, Billiot, F.H, Morris, K.F. An Investigation of the Effect of pH on Micelle Formation by a Glutamic Acid-BasedBiosurfactant.ColloidsInterfaces2024, 8, 38. https://doi.org/10.3390/colloids8030038

[7] Rani M, Weadge JT and Jabaji S (2020) Isolation and Characterization Of Biosurfactant-Producing Bacteria From Oil Well Batteries With Antimicrobial Activities AgainsFood-Borne and Plant Pathogens. Front Microbiol. 11:64. Doi: 10.3389/fmicb.2020.00064.

[8] Haytham M.M. Ibrahim, Characterization of biosurfactants produced by novel strains of Ochrobactrum anthropi HM-1 and Citrobacter freundii HM-2 from used Engine oil-contaminated soil. 31 December 2016.

[9] Nwaguma, I.V., Chikere, C.B. & Okpokwasili, G.C. Isolation, characterization, and application of biosurfactant by *Klebsiella pneumoniae* strain IVN51 isolated from hydrocarbon-polluted soil in Ogoniland, Nigeria. *Bioresour. Bioprocess.* **3**, 40, 2016. https://doi.org/10.1186/s40643-016-0118-4

[10] Kaur, H., Sangwan, S., Sharma, P., & Singh, S.. Biosurfactant production prospects of a Gram-negative bacterium-Klebsiella pneumoniae ssp. ozaenae

BK34. *Environmental monitoring and assessment*, *194*(5), 374, 2022. https://doi.org/10.1007/s10661-022-10033-8

[11] Fardami A.Y., Kawo A.H., Yahaya S., Ahmad S.A. and Ibrahim U. B. Stability and Optimization of Biosurfactant Production by Cloacae AYFI Enterobacter AJBAR Vol 1(1), 2022: 157-167, ISSN: 2811-2881

[12] P.Anna Joice and R.Parthasarathi. Optimization of biosurfactant production from Pseudomonas aeruginosa PBSC1. ISSN: 2319-7706 Volume 3 Number 9 (2014) pp. 140-151 http://www.ijcmas.com.

[13] Foukia E. Mouafia, Mostafa M. Abo Elsouda, Maysa E. Moharamb. Optimization of biosurfactant production by Bacillus brevis using Response surface methodology. 8 January 2016. www.elsevier.com/locate/btre.

[14] Chotard, M. Mounier, J. Meye, R. Padel, C. Claude, B. Nehme, R. Da Silva, D. Floch, S.L. Lucchesi, M.-E. Biosurfactant Producing Mucor Strains, Selection, Screening, and Chemical Characterization. Appl. Microbiol.2022, 2, 248–259. https://doi.org/10.3390/applmicrobiol2010018.

[15] Brinda C. M, R. Ragunathan and Jesteena Johney. Diversity And Distribution Of Potential Biosurfactant Producing Bacillus Sp MN 243657, GC – MS Analysis And Its Antimicrobial Study. 15-03-2023 http://dx.doi.org/10.13005/bbra/3088.

[16] Patowary K, Patowary R, Kalita MC and Deka S (2017) Characterization of Biosurfactant Produced during Degradation of Hydrocarbons Using Crude Oil As Sole Source of Carbon. Front. Microbiol. 8:279. Doi: 10.3389/fmicb.2017.00279.

[17] Huria Rizvi, Vikram Singh and Ashish. Screening and Characterization of Biosurfactant-producing Strains in Contaminated Soil 2024;18(2):1074-1084. https://doi.org/10.22207/JPAM.18.2.24.

[18] Walter Chinaka John, Innocent Okonkwo Ogbonna, Grace M. Gberikon, Charles Chidozie Iheukwumere. Evaluation of biosurfactant production potential of Lysinibacillus Fusiformis MK559526 isolated from automobile-mechanic-workshop Soil. 18 January 2021. https://doi.org/10.1007/s42770-021-00432-3.
Brazilian Journal of Microbiology (2021) 52:663–6.

[19] Badiaa Essghaier, Nesrine Mallat, Khaoula Khwaldia, Filomena Mottola, Lucia Rocco and Hédia Hannachi Production and Characterization of New Biosurfactants/Bioemulsifiers from Pantoea alhagi and Their Antioxidant, Antimicrobial and Anti-Biofilm Potentiality Evaluations. Laboratory of Mycology.17 February 2023.

[20] Ebtehag A.E. Sakr, Hala Abd Elmonem Ahmed, Feriala A.A. Abo Saif, International Journal of Biological Macromolecules Volume 170, Pages 94-106, 2021, https://doi.org/10.1016/j.ijbiomac.