Biosurfactant Production by *Pseudomonas aeruginosa* Strain LTR1 and its Application

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**Abstract:** On the circumstances of the growing market of biosurfactants all over the globe, the presented study focused on the characterization of biosurfactants produced by newly isolated and reported as biosurfactant producer strain *Pseudomonas aeruginosa* LTR1. Additionally, production media and physiological factors are optimized using a one factor at a time (OFAT) approach for maximum biosurfactant production. Its efficacy for antimicrobial and emulsification activities was determined. *P. aeruginosa* LTR1 utilized all the carbon sources provided, and a maximum of 9.5 g/L of biosurfactant was produced in soybean oil supplemented minimal salts medium (MSM). The critical micelle concentration (CMC) was determined as 12 mg/L and can reduce the surface tension of the medium from 72 mN/m to 31 mN/M. The biosurfactant was characterized by biochemical analysis and Fourier transform infrared spectroscopy (FTIR) and was not a protein in nature, possibly a glycolipid type of biosurfactant. It has shown the good emulsification activities for various hydrocarbon tested here. In addition, it acts as an antibiofilm agent with the minimum inhibitory concentration required for 50% biofilms reduction was 30.95 µg/mL suggesting the antimicrobial potential. The biosurfactant produced by *Pseudomonas aeruginosa* strain LTR1 has shown good surface-active properties, good emulsification, and antimicrobial activities, demonstrating its potential for application in various areas like the oil industry, especially in tank cleaning, bioremediation of spills at sea or soil, and candidates as antimicrobial.

**Keywords:** *Pseudomonas aeruginosa*; petrochemical contamination; biosurfactant; critical micelle concentration; antibiofilm

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**1. Introduction**

Biosurfactants are natural surface-active compounds produced biologically, mostly by bacteria, yeast, and fungi [1]. Glycolipids, liposaccharides, and proteins produced extracellularly are the biosurfactants that possess hydrophilic and hydrophobic domains [2]. Such properties render these molecules to reduce the surface and interfacial tension of compounds with different phases and form a microemulsion. Thus, biosurfactants and synthetic surfactants can act as excellent detergents, emulsifying agents, and dispersants [3,4].

Synthetic surfactants are organic compounds commonly used in various sectors such as soaps, detergents, shampoos, cosmetics, kinds of toothpaste, and drugs [5]. High production & energy costs, less solubility, charge, types, and physicochemical properties of synthetic surfactants are challenging criteria for selection and use for industrial purposes. The frequent uses of such chemical compounds increase their deposition at the user site and remain persistent
forever, increasing the environmental burden [6]. Moreover, toxicity is a major concern that may affect the flora and fauna of the ecosystem, disturbing the balance of the ecosystem [7]. On the other hand, biosurfactants are eco-friendly, biodegradable, better compatible, less toxic, and potentially active compared to synthetic surfactants [6]. Biosurfactants are a structurally diverse group of compounds that include simple fatty acids, glycolipids, peptides, lipids, siderophores, and polymeric surfactants [8]. Biosurfactants are now popular in various fields includes bioremediation, antimicrobials, food processing, and cosmetics. From an economic point of view, the biosurfactants market size was 1736.7 Million USD in the year 2019, and 5.5 % CAGR is expected for 2020–2026, with European countries as major customers [9].

Various anthropogenic activities are the major reason for hydrocarbon pollution in marine and terrestrial ecosystems [10]. Although hazardous and non-hazardous hydrocarbons pollute most of the oil filling station and highway side soil, such soil consortia are found rich in hydrocarbon degraders [11]. In natural settings, hydrocarbon degraders experience different interactions with other microorganisms, requiring more time for hydrocarbon degradation [12]. Interestingly, most isolated hydrocarbon degraders are efficient biosurfactant producers [13,14]. Producing biosurfactants by microorganisms using various substrates is more expensive; hence, various research groups commonly use agriculture products and waste as substrate [15]. Every year, tons of organic waste is generated worldwide from various industries, i.e., fruit processing industries, coffee processing industries, oil processing mills, and agronomic crops, which can be used as a renewable substrate source to produce biosurfactants [15]. Environment-friendly and surface activity make the biosurfactant an excellent agent for oil extraction, cosmetics, antimicrobial, and medicine [16]. Pseudomonas spp. usually found in oil-contaminated soil, oil-spill site, and hydrocarbon-containing soil can produce biosurfactants especially ionic glycolipids biosurfactants such as rhamnolipid [17]. In this research work, physiological conditions and important media components of MSM [18] are optimized for the production biosurfactant by P. aeruginosa strain LTR1 using the OFAT approach. Biosurfactant produced was further characterized, and its various activities, including surface emulsification activity against various hydrocarbons, were studied. Antimicrobial activities against the human pathogen Candida albicans were also studied.

2. Material and Methods

The P. aeruginosa strain LTR1 used in this study was previously isolated from the oil-contaminated soil sample from Latur, Maharashtra [19]. The strain was maintained on nutrient agar slant until further use. A single colony from 24 hrs old culture plate was inoculated in nutrient broth. 24 hrs old culture broth was used as an active culture for further uses.

2.1. Optimization of media components and physicochemical parameters for growth of biosurfactant producers.

Biosurfactant production is directly proportional to the growth of biosurfactant producers [20]. The maximum growth of bacterial isolate was optimized by using different parameters like carbon, nitrogen, temperature, pH, potassium dihydrogen phosphate, ferric chloride, and magnesium sulfate.

2.2. Screening of carbon and nitrogen sources.
The *P. aeruginosa* strain LTR1 was grown on various carbon sources such as glucose, soybean oil, kerosene, petrol, and diesel at 2 % (for glucose wt/v, rest v/v) concentration in MSM. Flasks were incubated at 37 °C for 12 to 120 hrs and 150 rpm on a rotary shaker incubator. The growth in each flask was determined by measuring optical density at 610 nm on a UV-double beam spectrophotometer (Systronics computer-based double beam spectrophotometer 2202). The maximum growth was considered positive for the utilization of a carbon source.

2.3. Optimization of temperature and pH.

To study the growth at different temperatures, *P. aeruginosa* strain LTR1 was inoculated in the MSM broth, which contains 2 % soybean oil. Culture inoculated media flasks were incubated at various temperature ranges for 12 to 120 hours at 150 rpm on a rotary shaker. The temperature range selected was 27 °C, 32 °C, 37 °C, 40 °C, and 45 °C. Similarly, after temperature optimization, pH optimization was carried out at 37 °C. The pH range used to optimize growth was 6, 6.5, 7, 7.5, 8, and 8.5. The growth in each flask was determined by measuring optical density at 610 nm on a UV-visible double beam spectrophotometer.

2.4. Optimization of potassium dihydrogen phosphate ferric chloride and magnesium sulfate.

For obtaining optimum growth of *P. aeruginosa* strain LTR1 at various concentrations of potassium dihydrogen phosphate (KH₂PO₄), ferric chloride (FeCl₃), and magnesium sulfate (MgSO₄) culture was inoculated in the mineral salt broth, which contains 2 % of soybean oil. Concentrations used were in the range of 1 to 5 g/L for KH₂PO₄, 10 to 60 mg/L for FeCl₃, and 100 to 500 mg/L for MgSO₄. Flasks were incubated at 37°C for 24 to 48 hrs at 150 rpm on a rotary shaker. The growth was determined by measuring optical density at 610 nm on UV-Vis double beam spectrophotometer [21].

2.5. Production of biosurfactant in batch culture.

MSM medium supplemented with 2 % Soybean oil as carbon source, includes 100 mg/L NH₄NO₃/(NH₄)₂SO₄, 3 g/L KH₂PO₄, 2 g/L K₂HPO₄, 200 mg/L MgSO₄, 10 mg/L CaCl₂ and 20 mg/L FeCl₃, was used as production medium for biosurfactant production. Five percent 24 hrs old *P. aeruginosa* strain LTR1 inoculum was transferred in a 1000 ml production medium, and the flask was incubated at 37 °C and 150 rpm for 3 days. The pH of the medium was initially adjusted to 7.0 ± 0.2 by 0.1 M HCl.

2.6. Biosurfactant extraction and recovery.

Extraction was carried out by the acid precipitation method. The bacterial cells were removed from the broth by centrifugation at 5000 rpm for 30 min. at 4 ºC. Acid precipitation was used to remove lipid and proteins, adding 6 M HCl to achieve a final pH of 2.0, and the flask was kept in a refrigerator at 4 ºC overnight. The white precipitate was collected, and the supernatant was discarded after centrifugation at 5000 rpm for 20 min. To extract the biosurfactant, the precipitate was dissolved in chloroform:ethanol (2:1) solvent system for 10 min. The organic phase was collected and concentrated by evaporation in a hot air oven at 45 ºC temperature yielding a viscous, honey-colored crude biosurfactant. Crude biosurfactant weight was recorded on the analytical balance. The concentrated crude biosurfactant was dissolved in methanol/chloroform and was used for further analysis [22].

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2.7. Characterization of biosurfactants.

The produced biosurfactant was analyzed for the qualitative detection of carbohydrate, protein, and lipid and was determined by the anthrone, Bradford & saponification method, respectively [23,24].

2.8. Purification of biosurfactant.

Analytical column chromatography was used to purify the biosurfactant. The column was packed by adding 50 gm slurry in 26 × 3.3 cm² glass chromatographic column of activated silica gel G-500 prepared in chloroform (CHCl₃). One-gram crude biosurfactant sample dissolved in 5 mL of chloroform was loaded onto the top of the prepacked chromatography column. The column was washed with chloroform to elute the neutral lipids. The various fractions of biosurfactant were collected stepwise by using chloroform/methanol in different ratio in sequence: 50:3 v/v (250 mL), 50:5 v/v (200 mL) and 50:50 v/v (100 mL) by maintaining a flow rate of 1 mL/min at room temperature. All the eluted fractions of biosurfactant were dried in a hot air oven at 45 °C to obtain the pure product.

2.9. Surface tension measurement.

The purified biosurfactant was diluted in deionized water, and the surface tension was measured using a Du Nouy ring type, Data physics (Angeltoni-DCAT11) tensiometer. Deionized water was used for standardization, and SDS was used as a standard surfactant. Biosurfactant was diluted in the range of 1.0 to 200 mg/L in distilled water. The average value of triplicate readings was considered the surface tension of respective solutions.

2.10. Determination of CMC.

Critical micelle concentration (CMC) of the biosurfactant was calculated by plotting surface tensions versus biosurfactant concentrations. Concentration at which no significant variation in surface tension was observed and considered CMC of the biosurfactant [25].

2.11. Fourier Transform Infrared (FTIR) analysis.

The column purified biosurfactant was characterized by Fourier Transform Infrared spectrophotometer (FTIR) spectroscopy to detect functional groups. The IR spectrum was recorded on Prestige-21 FTIR (Shimadzu, Japan) in the spectral region in the range of 400 to 4000 cm⁻¹ at a resolution of 2 cm⁻¹ using 0.2 mM KBr pellet method [24].


The emulsification activity of the biosurfactant produced by *P. aeruginosa* strain LTR1 was determined [26]. 3 mL of strain LTR1 grown supernatant was mixed with 3 mL of various hydrocarbons in separate tubes. Tubes were mixed vigorously mixed for 2 minutes and kept still for a further 24 hrs. Percent emulsification activity was determined by using the following formula:

\[ EI24\% = \frac{\text{Height of the emulsified layer}}{\text{total height of the liquid column}} \times 100 \]
2.13. Antibiofilm activity.

Antibiofilm activity of biosurfactant produced by LTR1 was studied against *Candida albicans* biofilms. In brief, 1×10⁶ cells/mL in phosphate-buffered saline (PBS) were distributed in each well of 96 well microtiter plates. The plate was incubated at 37 °C for 90 minutes on a rotary shaker incubator for adhesion. After completion of the incubation period, wells were washed with PBS. Various concentrations of biosurfactant ranging from 100 to 6.25 µg/ml in 200 µl of RPMI-1640 were distributed in prewashed cell adhered plate. The plate was incubated at 37 °C for 24 hrs and 120 rpm in a shaking incubator. After 24 hrs incubation, all the liquid media were removed. Wells were washed thrice with sterile PBS to remove unadhered cells/biomass. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay was performed to determine the biomass formed [27]. Light microscopic pictures were taken on Olympus inverted microscope (Model-CK40).


In triplicate, media component and physiochemical optimization, emulsification, and antibiofilm activity experiments were performed. Means and standard deviations are calculated and represented graphically. Shapiro-Wilk test was conducted to test non-normality. One-way ANOVA tests were performed at 0.05 p-value.

3. Results and Discussion

3.1. Screening of carbon and nitrogen substrate for the growth of biosurfactant producers.

There is a direct relation of growth with biosurfactant production [20]. Different carbon sources were screened in the present study to obtain optimum growth at various time intervals (Figure 1A and 1B). Four different carbon substrates such as petrol, kerosene, diesel, and soybean oil were taken for the study. Among all these, maximum growths were obtained in soybean oil as a sole carbon source in 72 hours incubation followed by petrol. The moderate growth was observed in diesel which showed maximum growth on the third day. The least growth was observed in kerosene oil at all different time points taken for the study.

3.2. Optimization of physicochemical parameters and metal constituents.

Temperature, pH, KH₂PO₄, K₂HPO₄, MgSO₄, CaCl₂ & FeCl₃ concentrations were optimized to obtain maximum growth of *P. aeruginosa* strain LTR1 (Figure 1). Effect of temperature on the growth of *P. aeruginosa* strain LTR1 was studied in the range of 27 °C to 45 °C. The maximum growth increment was observed at 37 °C in soybean oil within 24 hrs. The trend continued up to 72 hrs and started declining after that (Figure 1C). In the case of hydrogen ion concentration, *P. aeruginosa* strain LTR1 showed maximum growth at pH 7 in soybean oil as the sole carbon source within 72 hrs (Figure 1D). KH₂PO₄ and K₂HPO₄ are the best sources of potassium and phosphate, which show an effect on the growth and synthesis of biosurfactants. *P. aeruginosa* LTR1 showed maximum growth at 2 g/L and 3 g/L concentrations of KH₂PO₄ and K₂HPO₄, respectively, in the flask containing soybean oil as sole carbon source and pH 7.0 incubated at 37 °C, 150 rpm in shaking incubator (Figure 1E). Increasing the concentration of KH₂PO₄ and K₂HPO₄ increases the growth of organisms till the concentration reaches 2 g/L and 3 g/L, respectively; beyond that, increasing concentration decreases the growth of the microorganism. The divalent cations such as Mg²⁺ and Ca²⁺ affect
the emulsification activity of the biosurfactant synthesized by *P. aeruginosa*. Increasing concentration of Mg$^{++}$ from 1.1 mM to 2.5 mM concentration increased emulsification activity [28]. Furthermore, MgSO$_4$ as a divalent cation and trace element affects growth and emulsification activity [29]. Maximum growth was observed at 200 mg/L concentration of MgSO$_4$ in the flask containing soybean oil. Increasing the concentration of MgSO$_4$ increases the growth of organisms till the concentration reaches 200 mg/L, and beyond that, increasing concentration decreases the growth (Figure 1F). Similarly, CaCl$_2$ also showed a concentration-dependent change in the growth. Maximum growth was observed at 10 mg/mL at 72 hours. Later concentrations of CaCl$_2$ showed a decrease in the growth. Ferric chloride as a source of iron is also one of the important constituents of MSM and essential for biosurfactant production media[30]. The optimum concentration of FeCl$_3$ required for maximum growth was 20 mg/L after 72 hours incubation containing soybean oil as sole carbon source, pH 7 at 37 °C (Figure 1G).

**Figure 1.** Figure: effect of carbon source, Temperature pH, and various metal sources on growth of biosurfactant producing strain LTR1. Growth at A: various carbon sources; B: Temperature; C: pH; D: KH$_2$PO$_4$ E: K$_2$HPO$_4$; F: MgSO$_4$; G: CaCl$_2$; H: FeCl$_3$, p ≤ 0.05.

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3.3. Production of biosurfactants by batch culture.

In the optimized production medium *P. aeruginosa* strain, LTR1 showed pigmented and turbid growth after 48 hours of incubation. About 9.5 g/L of biosurfactant yield was obtained within 72 hours.

3.4. Biochemical characterization of biosurfactant.

The extracted biosurfactant was biochemically characterized for detecting carbohydrates, protein, and lipids using qualitative tests. Anthrone and saponification tests for carbohydrates and lipids, respectively, were positive for the extracted biosurfactants. Bradford test performed for protein was negative. Biochemical tests indicate that the biosurfactant produced by *P. aeruginosa* strain LTR1 is glycolipid. Most of the biosurfactant glycolipids produced by *Pseudomonas* are rhamnolipid. So, we further analyzed the biosurfactant for rhamnolipids.

3.5. Purification of biosurfactants by column chromatography.

The biosurfactant produced by *P. aeruginosa* strain LTR1 was extracted by acid precipitation method, and the crude extract was subjected for further purification using silica gel column chromatography. The fraction of rhamnolipid eluted at 50:50 v/v methanol: chloroform was restored. The purified biosurfactants were light yellow to brownish and viscous liquids with solubility in most organic solvents but sparingly soluble in water and methanol. The recovered biosurfactant was dried by using a rota-evaporator to the powder.

3.6. Identification of functional group by FTIR.

FTIR is a powerful technology for the functional group's determination of chemical compounds. Purified biosurfactant was analyzed for functional group detection using FTIR. The significant peaks were observed at 3240 cm\(^{-1}\), 2923 cm\(^{-1}\), 2854 cm\(^{-1}\), and 1745 cm\(^{-1}\) vibrations indicating stretching for –CH and –CH\(_2\). Vibration at 2923 cm\(^{-1}\) and 2854 cm\(^{-1}\) indicates –CH\(_3\) stretching. In addition, strong stretching of –C=O of the ester carbonyl group was observed at 1745 cm\(^{-1}\). The FTIR spectra of biosurfactants from *P. aeruginosa* LTR1 also revealed the presence of lipid moiety in the purified glycolipids showing peaks at 1458 cm\(^{-1}\), 1378 cm\(^{-1}\), 1147 cm\(^{-1}\), 1073 cm\(^{-1}\), and 1013 cm\(^{-1}\). The intensity band in the region of 1455–1386 cm\(^{-1}\) shows bending of the hydroxyl (–OH) group, which reflects the presence of the carboxylic acid as a functional group in the compound [31]. Similarly, the biosurfactant showed an intensity band at 1458 cm\(^{-1}\) indicated bending of the hydroxyl (–OH) group, which reflects the presence of the carboxylic acid functional group in the compound. The band in the region of 1378 cm\(^{-1}\) was the result of deformations and bending vibrations of –C-CH\(_3\) and –C-CH\(_2\) groups (Figure 2).
3.7. Surface tension & determination of CMC.

The reduction in surface tension measurement and determination of critical micelle concentration (CMC) of crude biosurfactant was carried out to measure extracted biosurfactants' tension-active properties and effectiveness. The biosurfactant obtained from \textit{P. aeruginosa} strain LTR1 was able to reduce the surface tension of water from 72 to 31 mN/m. The point of deflection obtained from the surface tension reduction curve at the lowest concentration of biosurfactants was 12 mg/L called critical micelle concentration (CMC). Further increase in the concentration of biosurfactants showed a very negligible impact on the lowering of surface tension (Figure 3).

3.8. Emulsification activity.

Emulsification activity of the biosurfactant produced in the supernatant culture of \textit{P. aeruginosa} strain LTR1 determined for kerosene, diesel, petrol, and soybean (Figure 4). The supernatant containing biosurfactant, emulsification index for soybean was maximum (74.66 ± 3.05) and minimum for diesel (38.33 ± 7.03). Emulsification indices for kerosene and petrol were 50 ± 5.56 and 48 ± 3, respectively (Figure 4). A higher emulsification index for a biosurfactant of the bacteria against hydrocarbon indicates the stronger hydrocarbon-degrading capacity of bacteria [32].

![Figure 2](https://doi.org/10.33263/BRIAC131.010)

\textit{Figure 2}. Determination of surface tension & CMC of biosurfactant produced by \textit{P. aeruginosa} strain LTR1 in optimized production media containing Soybean oil as sole carbon source.

![Figure 3](https://biointerfaceresearch.com/)

\textit{Figure 3}. FT-IR spectra of biosurfactant produced by \textit{P. aeruginosa} strain LTR1 using Soybean oil as the sole carbon source.
3.9. Antibiofilm activity of purified biosurfactant.

Biofilms are challenges for clinicians and researchers due to their high resistance against antimicrobials, especially antibiotics [33,34]. Biosurfactants have shown their potential as antimicrobials against drug-resistant microorganisms [35]. Antibiofilms activity of purified biosurfactant was determined by broth dilution method and estimated by MTT assay. There was a concentration-dependent decrease in biofilm formation when treated with various concentrations of biosurfactant (Figure 5 and 6). The minimum inhibitory concentration was recorded to be 30.91 µg/mL. The inhibition concentration was higher than other standard antifungals like caspofungin (0.25 µg/mL) and other antifungals [36], but they can be potential drug development candidates.

Figure 4. Emulsification activity of a supernatant culture of *P. aeruginosa* strain LTR1 against various hydrocarbons, *p* ≤ 0.05.

Figure 5. Light microscopy picture of biofilms treated with different concentrations of purified biosurfactant produced by *P. aeruginosa* strain LTR1. A: 100 µg/mL, B: 50 µg/mL, C: 25 µg/mL, and D: Control (no biosurfactant).
Various carbon sources such as olive oil and distillery wastes, processed wastewater from food industries, kerosene, diesel, petrol, n-hexadecane, paraffin oil, whey, molasses, palm oil, canola oil, vegetable oil, and corn oil refinery wastes were successfully used to produce biosurfactant [37]. Due to their easy availability throughout the year, easy transportation, and low cost, these raw materials locally grasped more attention to use as a substrate for the production of biosurfactants [38]. The soybean is one of the major vegetable oil crops produced worldwide, and supply is more than that the demand [39]. It is a rich source of unsaturated fatty acid consisting of oleic, linoleic, and linolenic acid [40]. India's Marathwada and West Maharashtra region has a high cropping pattern of soybean as a major cash crop cultivated to produce soybean oil [41]. Here in this research communication thus we used soyabean oil as one of the carbon sources and kerosene, petrol, and diesel.

*Pseudomonas* species have been reported as a prominent biosurfactant producer and produce glycolipids type biosurfactants, mainly rhamnolipid. Functional group analysis by FTIR and biosurfactant characterizations studies indicate that isolated biosurfactant is not a protein in nature. NMR and MS studies may explain the exact structure of the glycolipid type of biosurfactant. Mercade *et al.* reported the 6.4 g/L of rhamnolipid biosurfactant production from *Pseudomonas* spp. JAMM using olive oil mill effluents which were extracted by acid precipitation method [38]. Rahman *et al.* reported 4.31 g/L rhamnolipid production using soybean oil from *P. aeruginosa* DS10-129. With 12 days of fermentation [42]. In the comparative study of the production of biosurfactants using various vegetable oils at 2 % concentration such as olive oil, palm oil, and coconut oil in defined mineral salt medium from *P. aeruginosa* A41 strains [43]. The optimum yields of 6.58 g/L, 2.91 g/L, and 2.93 g/L biosurfactants production were reported in 2 % olive oil, palm oil, and coconut oil, respectively. In rhamnolipid production by *P. aeruginosa*, DS10-129 exploited soybean oil as a substrate with 12 days of incubation. There was no significant growth detected within three days of incubation. Later, a progressive increase in growth was observed up to 12 days. Later, there was no significant increase in the growth and production of rhamnolipid [42]. This exhibited that biosurfactants were produced as secondary metabolites.

In comparison to *P. aeruginosa* DS10-129 to produce biosurfactants using soyabean oil, *P. aeruginosa* LTR1 were the best candidates having a one-day lag phase with a higher growth within 72 hrs. Biosurfactant yield by *P. aeruginosa* strain LTR1 was sufficiently more.
and showed a reduction of surface tension of water. The biochemical constituent of biosurfactant produced by *P. aeruginosa* strain LTR1 is Rhamnolipid type. Rhamnolipids are glycolipids shown to be efficient metal chelating agent that interacts with metals through a polar glycosidic bond [44]. In the rhamnolipid treated calcareous soil, biomass production was increased as it helps in the absorption of Zinc by the plant [45]. The chelating metal property of the biosurfactants has made these molecules a potential candidate for heavy metal remover from water and soil [46]. Biosurfactants are also have been utilized for enhanced crude oil extraction transportation and recovery [8]. Biosurfactant produced by *P. aeruginosa* strain LTR1 showed emulsification activity in the range of 38 to 74 against vegetable oil and petrochemical hydrocarbon, suggesting its use in oil refiners and bioremediation of soil and water bodies contaminated by oils due to spillage. Antibiofilm activity at 30.95 µg/mL is an indicative figure as a potential candidate for antimicrobial drug development. Thus, the rhamnolipid produced from hydrocarbonoclastic bacteria *P. aeruginosa* strain LTR1 with promising tensioactive properties and antimicrobial may find applications in various industries.

4. Conclusions

The biosurfactant could be produced from various carbon compounds, including an agricultural product like soybean oil using *P. aeruginosa* LTR1. Physiochemically optimized MSM medium showed 9.5 g/L of biosurfactant production within 72 hours. The biochemical characterization of biosurfactant revealed the presence of carbohydrate and lipid, which had tension-active properties of 31 dyne/cm with a critical micelle concentration of 12 mg/mL. Culture supernatant of *P. aeruginosa* strain LTR1 showed emulsification activity in the range of 38 to 74 against vegetable oil and petrochemical hydrocarbon, suggesting its use in oil refiners and bioremediation of soil and water bodies contaminated by oils due to spillage. Antibiofilm activity at 30.95 µg/mL is an indicative figure as a potential candidate for antimicrobial drug development. Thus, the rhamnolipid produced from hydrocarbonoclastic bacteria *P. aeruginosa* LTR1 with promising tensioactive properties and antimicrobial may find applications in various industries.

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**Conflict of interest**

The authors declare no conflict of interest.

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