Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/herncx95

EXPLORING THE POTENTIAL OF BIOSURFACTANT-PRODUCING ENDOPHYTIC *PSEUDOMONAS AERUGINOSA* SF1R IN MEDICAL THERAPEUTICS

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Abstract
The emergence of antibiotic resistance and demand for eco-friendly therapeutic agents have intensified interest in microbial biosurfactants with biomedical applications. This study explores the potential of *Pseudomonas aeruginosa* SF1R, an endophytic strain isolated from *Mangifera indica*, as a biosurfactant producer with promising therapeutic relevance. SF1R isolate was screened and selected for biosurfactant production followed by medical applications including antibacterial, antibiofilm and antioxidant activity. SFIR strain produced crude biosurfactant product of approximately 1117 mg/L with functional activities like 60 mm oil displacement activity, 38% emulsification index and strong activity in terms of drop collapse test. Biosurfactant produced by SF1R isolate showed maximum antibacterial activity (41 ± 1.5 mm) against *E.coli* followed by *S. typhae* (35.5 ± 0.7 mm). The biosurfactant resulted in 57% inhibition of the biofilm produced by S. *aureus,* followed by 16% inhibition of the biofilm of *E. coli.* Likewise, in biofilm eradication, 55% was observed in *S. aureus* followed by *E.coli* (28%). However, antioxidant activity in the form RSA of biosurfactant was observed as 60%.

Key words: Biosurfactant, Antibacterial, antioxidant, Antibiofilm, *Pseudomonas aeruginosa*

Introduction:

Endophytes are microbe that reside inside plant tissues without causing any negative physiological and pathological alteration in the host plant. They make symbiotic interactions with plants and benefit the host plant whole the time or under specific biotic and abiotic stress circumstances (Pisarska *et al.,* 2014). Some imported endophytes are *Acinetobacter, Actinomyces, Aeromonas, Agrobacterium, Alcaligenes, Bacillus, Corynebacterium, Enterobacter, Mesorhizobium, Pseudomonas, Rhodococcus, Rhizobium, Rhizobacter, Rhizomonas, Sphingomonas, Spirillum, Streptomyces, Sphingobacterium* and *Stenotrophomonas* (wu *et al.,* 2012; Lodewyckx *et al.,* 2002).

Endophytes frequently produce secondary metabolites having bioactive characteristics. These Secondary metabolites play an important role in medical sciences, agronomy, biodegradation and food sciences (Mishra *et al.,* 2018). Biosurfactants are considered as significant secondary metabolites from endophytic microbes (Singh *et al.,* 2017). Biosurfactants are biological molecules produced by various microbes like fungi, bacteria and yeast. These biomolecules contain hydrophilic as well as hydrophobic moieties. The hydrophilic part includes a head which is polar containing mono, di, oligo or polysaccharides and polypeptides, while the hydrophobic part is the tail which contains

saturated/unsaturated hydrocarbon chains and hydroxylated fatty alcohols/fatty acids (Phulpoto *et al.,* 2024).

Biosurfactants are classified into different groups/classes, 1) glycolipids which include sorpholipids, rhamnolpids and liposomes, 2) lipopolypeptides that include surfactin and serawetten vicosin, 3) fatty acids including phospholipids and neutrolipids, 4) polymers that include emulsan, liposan, alasan and others.Further, biosurfactants are considered better than synthetic surfactants due to their eco-friendly nature, low toxicity, high biodegradability, increased surface activity and stability in extreme circumstances like temperature, pH and salinity (Abbot et al ., 2022; Sun *et al.,* 2019).

Diverse biosurfactants molecules are reported from microbial diversity in previous studies, which are categorized by the microbial origin and biochemical structure like Rhamnolipids from *P. aeruginosa,* Sophorolipids from *Candida bombicola*, Mannosylerythritol lipids and Cellobiose lipids from fungi and yeast such as *C. Antarctica*, Trehalose lipids and Succinyl-trehalose lipids from *Glomerella cigulata*, Xylolipids from *Lactococcus lactis,* Emulsan from *Acinetobacter calcoacetius*, and Oligosacchride lipids from *Tsukamurella sp.* (Sotirova *et al.,*2009; Kitamoto *et al.,* 2002). The important biosurfactants having antimicrobial potential include rhamnolipids, lipopolypeptides, Fengycin, surfactin, Iturin, lichenysin, pumliacidin, and Bacillomycins (Das *et al.,* 2014; Vater *el al.,* 2002).

Microbial surfactants are also known for their medical applications including antiviral, antifungal, antibacterial, anti-inflammatory, antitumor growth, anti-asthma, immune- modulator and antiadhesive characteristics (Sharma &Singh 2014). Moreover, biosurfactants also have an essential role in quorum sensing, cell adhesion, cell aggregation, production of anti-biofilm and antimicrobial compounds, and solubility of water-insoluble compounds (Gudiña *et al.,* 2010). Rhamnolipid from *Pseudomonas aeruginosa* is reported for antifungal activity. Pontifactin from *Pontibacter korlensis* for antimicrobial and antibiofilm activity. Lipopetides from Bacillus sp., for antiviral and anti-cancerous activity. Soprholipid form *Rhodotorula bogoriensis* is also reported for antimicrobial property (Balan *et al.,* 2016; Coronel-León *et al.,* 2015; Goswami *et al.,* 2014; Donio *et al.,* 2013).

The current study focused on the production of biosurfactants from endophytic *Pseudomonas aeruginosa* and the potential application of biosurfactants in medical sciences including antimicrobial, anti-biofilm and antioxidant activity.

Material and Methods

Isolation and characterization of biosurfactant producing endophytic *Pseudomonas aeruginosa* SF1R

Surface sterilization of plant samples was done according to standard protocols. Endophytic bacterial isolation was done according to Nair *et al.,* (2014) technique. Plant parts (Stem, leaves, fruit & root) were washed with tap water and chopped into slices with the sterilized scalpel. Surface sterilization was carried out using 70% ethanol and 0.1% mercuric chloride ($HgCl_2$). Aseptically chopped slices were inoculated on the surface of Nutrient Agar plates and plates incubated for 24 to 48 hours at 37 ºC. Different endophytic bacterial colonies were sub-cultured to obtain pure cultures. Pure cultures were subjected to cultural morphological and biochemical characterization according to the standard protocol of Berg's manual. Molecular identification was carried out using 16sRNA sequence homology.

Screening of biosurfactant-producing endophytic microorganisms

Screening of biosurfactant-producing endophytic bacteria was carried out by the method of Phulpoto *et al.,* (2020) using Nutrient broth supplemented with 1% Olive oil as an oil source. Fresh cultures having Optical density =1 at 600 nm were inoculated in flasks. Flasks were incubated at 150 rpm at 37°C for 5 days. Samples were centrifuged at 5000 rpm for 15 minutes to obtain cell-free supertenant (CFS). CFS was then processed for screening of biosurfactants.

Emulsification activity

Emulsification activity was evaluated by protocol of Phulpoto *et al.,* (2020). 1mL of CFS was taken in the test tube, and 1mL of kerosene oil was added and mixed vigorously. The mixture was vortexed for 2 min. Emulsification was recorded after 24 hours. The emulsification index (%) was calculated using the formula.

$$E\ 24\ (\%) = \frac{Height\ of\ Foam}{total\ height\ of\ mixture} \times 100$$

Oil displacement method

Oil displacement activity (ODA) was done using protocols reported in previous literature (Phulpoto *et al.,* 2020). Approximately 200 µL of used engine oil was placed on the surface of the Petri plate containing 30 mL of distilled water. Then, 20 µL of CSF was dropped on oil-coated thin film with the help of a pipette. The zone of oil displaced due to CSF was measured in millimetres (mm).

Drop collapse method

Drop collapse activity (DCA) was performed according to Fooladi *et al.,* (2016). A thin layer of oil was placed on the paraffin coating film. The film was left for equilibration for 1–2 hours. About 10 µL CFS was applied to film for 1 to 2 minutes, to check the potential of biosurfactant. The collapsed drops suggested the presence of biosurfactants in the CFS.

Batch fermentation for biosurfactant production and recovery of biosurfactants

The bacterial isolates were streaked on fresh nutrient agar plates supplemented with antifungal antibiotics and were incubated for 24 h at 37°C. The fresh culture was inoculated in a 100 ml conical flask containing 50 ml of nutrient broth and was incubated in a shaker at 150 rpm at 37 °C for 24 h. The Minimal Salt Medium (MSM) was prepared for the growth culture reported by Shah *et al.,* (2016) containing 2% olive oil sole carbon source. The 2 ml of prepared inoculum were transferred into the MSM medium and culture broths were incubated at 150 rpm for 7 days at 37°C batch. After seven culture broth was centrifuged for 15 minutes at 5000 rpm to collect cell-free CFS for the extraction of biosurfactants.

The recovery of biosurfactants was done using acid precipitation as described (Donio *et al.,* 2013). First, HCl was added to CSF to make pH up to 2.0 and was incubated overnight at 40 ºC. An equal volume of ethyl acetate was added. White precipitates were separated using a funnel and kept in a petri plate at 45 ºC to obtain crude product. Crude biosurfactant was used for further analysis (E 24%, ODA, DCA) and medical applications.

Medical applications of biosurfactants

Antibacterial activity

Antibacterial assay of biosurfactants was performed against 04 clinical isolates (*Staphylococcus aureus, Bacillus spp, E. coli & Salmonella typhi)*, according to Sana *et al.,* (2018). Fresh cultures (OD=1 at λ= 600 nm) were streaked on the surface of Muller Hilton Agar plates. Wells of 6mm were done with the help of a borer then, 20 µl of liquid biosurfactant was transferred to wells and named accordingly. Plates were incubated at 37 ºC for 24 to 48 hours. The zone of inhibition (mm) was measured after 48 hours.

Antibiofilm activity

Antiobiofim activity of biosurfactants was performed according technique described by Wolfmeier *et al.,* (2018). Biofilm-producing bacteria, *Staphylococcus aureus* and *E. coli* were obtained from PGRL, Institute of Microbiology. Antiobiofilm activity was checked by 02 methods.

Biofilm inhibition

Pure cultures of biofilm-forming bacteria (50 µl) were transferred to 96 well microtiter plates followed by adding approximately 50 µl of biosurfactants to inoculated wells. The plate was incubated at 37 ºC for 48 hours & washed with Phosphate buffer saline (PBS) buffer. Approximately 100 µl of 0.1 % crystal violet was added to wells and again plate was incubated for 30 mins followed by washing with PBS buffer. Finally, 100 µl of ethanol was added to the wells, and the plate was examined in the microtiter plate reader at λ=570nm.

Biofilm eradication

Pure cultures of biofilm-forming bacteria (50 µl) were transferred to 96 well microtiter plates. The plate was incubated at 37 ºC for 48 hours & washed with Phosphate buffer saline (PBS) buffer. Approximately 50 µl of biosurfactants was added to inoculated wells and the plate was incubated for 30 min at 37 ºC followed by washing with PBS. 100 µl of 0.1 % crystal violet was added to wells and again plate was incubated for 30 mins followed by washing with PBS buffer. Finally, 100 µl of ethanol was added to wells and the plate was examined in the microtiter plate reader at λ=570nm.

Antioxidant Activity

Antioxidant activity/Radical scavenging activity (RSA) of biosurfactants was performed, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method reported by Ohadi *et al.,* (2017). Initially, DPPH (0.1mM) was prepared in Ethanol. Then, 40 µL sample was added in 2.960 mL of DPPH in test tubes and tubes were Incubated at 37 ºC in dark conditions for 30 mins. After that absorbance was recorded at λ=570nm using Spectrophotometer. Radical Scavenging activity (%) was calculated using the formula.

$$\textbf{RSA (\%)} = \frac{Ab\ (control) - Ab\ (sample)}{Ab\ (Control)} \textbf{x100}$$

Statistical analysis

All the experiments related to biosurfactants, functional screening i.e. emulsification, oil displacement biosurfactant production and application activities were performed in triplicates. The mean ± standard deviation (SD), LSD were calculated using Statistix 8.0.

RESULTS AND DISCUSSION

Isolation and characterization of endophytic *Pseudomonas aeruginosa* SF1R

A total of 45 endophytic bacteria were isolated from plants and were screened for biosurfactant production. The potential isolate in biosurfactant production and medical application was SF1R, which was isolated from the root part of *Mangifera indica* (Mango) and the isolate was identified as *Pseudomonas aeruginosa*. The complete colonial, morphological, microscopic and biochemical characterization is listed in table 1. However phylogenic tree is shown in figure 1.

The endophytic isolates SF1R was *Pseudomonas aeruginosa*. Our finding resembles different researchers who also reported *Pseudomonas aeruginosa* and other species like *Acinetobacter, Actinomyces, Agrobacterium, Alcaligenes, Bacillus, Corynebacterium, Mesorhizobium, Pseudomonassp, Rhodococcus, Rhizobium, Rhizobacter, Rhizomonas, Sphingomonas, Sphingobacterium, Stenotrophomonas* (Pisarska *et al.,* 2014; Wu *et al.,* 2012; Lodewyckx *et al.,* 2002).

Table 1. Morphological and biochemical characterization of *Pseudomonas aeruginosa* SF1R.

Tests	Results
Colonial characteristics	
Colonies, Pigment on nutrient agar	Circular, Raised, Smooth margin Diffusible green pigment turning blue green
Microscopy	
Gram stain	Negative
Motility	Motile
Cell shape and arrangement	Small rods, Mostly single
Biochemical characteristics	
Oxidation/fermentation test	Strictly aerobe
Citrate utilization	+
Catalase	+
Oxidase	+
Indol production	-
Methyl red fermentation	-
H2S production	+
Starch hydrolysis	+
Lipid hydrolysis	+
Gelatin liquefaction	+
lood hemolysis	+

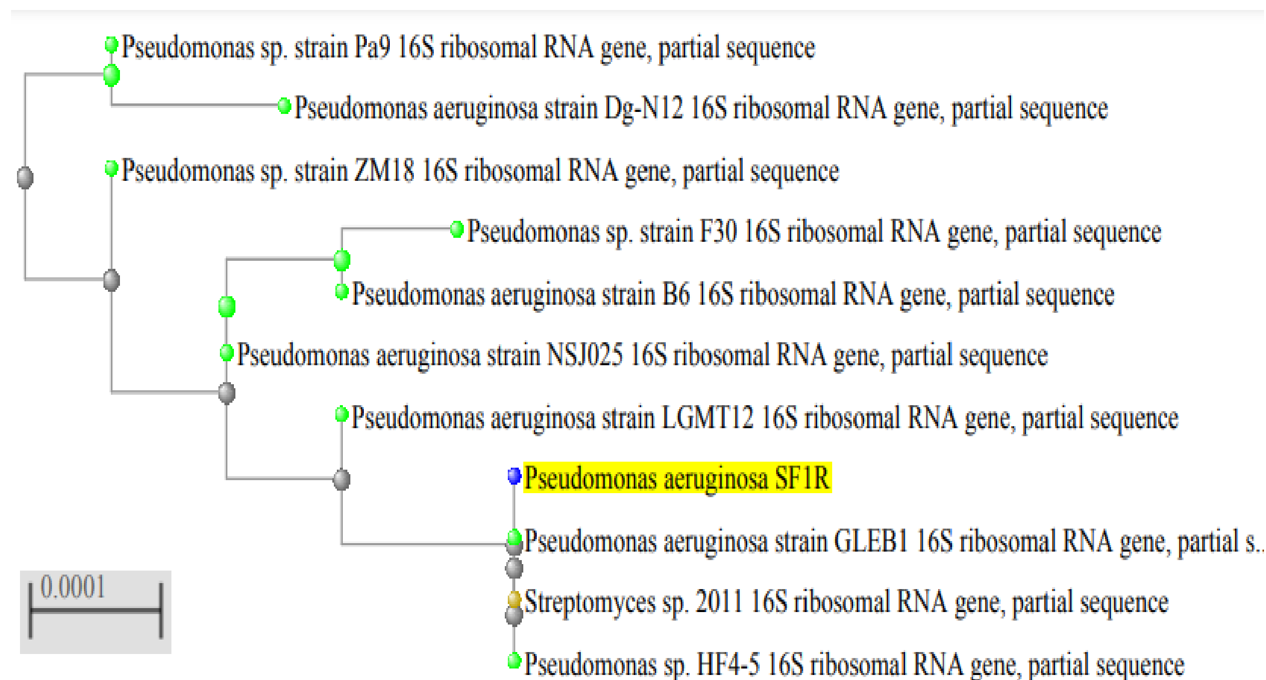

Figure 1. Phylogenic tree of *Pseudomonas aeruginosa* SF1R.

Screening and production of biosurfactants

Pseudomonas aeruginosa SFIR was initially among the top isolates during screening of biosurfactant production. In the batch fermentation for biosurfactant production, SFIR strain produced crude biosurfactant product of approximately 1117 mg/L with functional activities like 60 mm oil displacement activity, 38% emulsification index and strong activity in terms of drop collapse test as shown in table 2.

Our findings correlate to results of different scientist who reported average crude product of *Pseudomonas aeruginosa* and other microbes as, *Pseudomonas aeruginosa* (300–2000 mg/L),

Bacillus subtilis (500 to 2000 mg/L), *Rhodococcus erythropolis* (1–3 mg/L), *Yarrowia lipolytica* (1–3 mg/L), *Candida bombicola* (500 to 800 mg/L) (Mahmoud *et al.,* 2024; Morita *et al* .,2013; Abdel-Mawgoud *et al.,* 2011; Van Bogaert *et al.,* 2007).

Table 2. Biosurfactant production and functional activities of *Pseudomonas aeruginosa* SF1R.

Parameter	Result
ODA	60 mm
E 24(%)	38%
DCT	+++ (Strong activity)
Crude Product	1117 mg/L

Medical applications of biosurfactants

Biosurfactant produced by SF1R isolate showed maximum antibacterial activity (41 ± 1.5) against *E.coli* followed by *S. typhae* (35.5 ± 0.7), *S. aureus* (6.5 ± 1) and *Bacillus Sp* (6 ± 0.7). In the case of biofilm inhibition, the biosurfactant resulted in 57% inhibition in biofilm produced *S. aureus* followed by 16% inhibition in the biofilm of *E.coli.* Likewise, in biofilm eradication by biosurfactant, 55% was observed in *S. aureus* followed by *E.coli* (28%). However, antioxidant activity in the form RSA of biosurfactant was observed as 60%. The summarized results of antibacterial activity, biofilm inhibition & eradication and antioxidant activity are listed in table 3 respectively.

Table 3. Medical applications of biosurfactant produced by *Pseudomonas aeruginosa* SF1R

Parameter	Result
Antibacterial activity (Zone in mm)	
S. aureus	6.5 ± 1
Bacillus Sp	6 ± 0.7
S. typhae	35.5 ± 0.7
E.coli	41 ± 1.5
Biofilm inhibition	
S. aureus	57%
E.coli	16%
Biofilm eradication	
S. aureus	55%
E.coli	28%
Antioxidant activity	
radical scavenging activity (RSA)	60%

Biosurfactant/biosurfactant-producing endophytic microbes harbor different applications including medical (antibacterial, antibiofilm, antioxidant), Some other scientists also reported antioxidant, anti-cancerous, anti-inflammatory, antiviral, antifungal, antibacterial, anti-inflammatory, antitumor growth, anti-asthma, immune-modulator and antiadhesive characteristics aliphatic hydrocarbons (Phulpoto *et al.,* 2024; Gaur *et al.,* 2022; Ghasemi *et al.,* 2019; Das *et al.,* 2014; Burch *et al.,* 2011).
Balan *et al.,* (2016) reported antimicrobial and antibiofilm activity of biosurfactants, Wei *et al.,* (2004) reported Serrawettins role reduction in surface tension, Coronel-León *et al.,* (2015) reported the application of lipopeptides in cosmetics. Goswami *et al.,* (2014) reported the antifungal activity of rhamnolipids. Janek *et al.,* (2012) reported the antiadhesive activity of biosurfactants. Alej *et al.,* (2011) reported the anticancer role of sorpholipids. Rau *et al.,* (2005) reported antioxidant activity of glycolipids.
In our findings, maximum antimicrobial activity was observed against *E.coli, S.typhae*, *S.aureus* and *Bacillus sp* Maximum antioxidant (>50%) activity was observed. Biofilm inhibition/eradication (*E. coli* & *S. aureus*), maximum activity (>50%) was shown. Our results correlate with others who

Scientists reported rhamnolipid biosurfactants produced by *Pseudomonas* strain inhibited both Gram-positive and Gram-negative bacterial strains tested (i.e. *E. coli, B. subtilis, S. aureus* and *S. epidermidis*) (Das *et al.,* 2014). In another study reported by Dusane *et al.,* (2011), glycolipid showed antibacterial and anti-adhesive activity and inhibited the biofilm formation of the pathogens *C. albicans* and *P. aeruginosa*, as well as the marine biofouling bacterium *Bacillus pumilus*.

Conclusion

Pseudomonas aeruginosa SF1R exhibits strong biosurfactant production with notable antimicrobial, anti-biofilm, and antioxidant properties, alongside low cytotoxicity. Its endophytic origin enhances its therapeutic potential, making it a valuable candidate for developing sustainable medical treatments. Further in vivo studies are essential to validate its clinical applicability and formulation potential.

Acknowledgment

Authors acknowledge the professors, scientists, researchers and friends who helped in conducting this research.

Conflict of Interest

Authors declare that there was no any conflict of interest

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