chemistry proceedings

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Proceeding Paper

Screening of the Antibacterial Potential of the Biosurfactant Produced by *Pseudomonas fluorescens* ICCF 392 Against *Bacillus* sp. †

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† Presented at the 29th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-29); Available online: https://sciforum.net/event/ecsoc-29.

Abstract

Biosurfactants are amphiphilic biocompounds produced by microorganisms, recognized for their surface-active properties and broad biotechnological applicability. The rising concern over antimicrobial resistance and environmental impact of synthetic chemicals has increased the demand for natural and eco-friendly alternatives. Biosurfactants, due to their unique chemical structure and multifunctional properties, have emerged as promising candidates in this regard. These compounds have gained increasing attention due to their biodegradability, low toxicity, and potential to replace synthetic surfactants in various industries. In particular, their antimicrobial properties make them promising agents for applications in food safety, pharmaceuticals, and environmental protection. *Pseudomonas fluorescens*, a well-known biosurfactant-producing bacterium, has been extensively studied for its capacity to produce rhamnolipids with antimicrobial activity. Therefore, this study aimed to assess the antibacterial potential of the biosurfactant synthesized by *Pseudomonas fluorescens* ICCF 392 against *Bacillus cereus*, a Gram-positive bacterium frequently associated with foodborne illnesses. The biosurfactant was obtained through submerged fermentation and partially purified. The antibacterial activity was evaluated using the agar well diffusion method, which revealed a clear zone of inhibition measuring 20 mm in diameter. These findings indicate that microbial biosurfactants can serve as effective and sustainable alternatives to conventional antimicrobial agents. Further studies will focus on detailed characterization of the biosurfactant, its spectrum of activity, and potential formulation in various delivery systems.

Keywords: microbial surfactant; *Pseudomonas fluorescens*; antibacterial activity; *Bacillus cereus*; biotechnological applications

Academic Editor(s): Name

Published: date

Citation: Stoica, R.M. Screening of the Antibacterial Potential of the Biosurfactant Produced by *Pseudomonas fluorescens* ICCF 392 Against *Bacillus* sp. *Chem. Proc.* **2025**, *volume number*, x. https://doi.org/10.3390/xxxxx

1. Introduction

Microorganisms from diverse genera, including *Pseudomonas*, *Bacillus*, and *Candida*, are known to produce biosurfactants with important surface-active properties. Within this group, species belonging to *Pseudomonas* have been widely investigated because of their ability to produce rhamnolipids, a class of biosurfactants with versatile chemical structures and a broad spectrum of biological activities. Compared with synthetic surfactants, microbial biosurfactants are generally more environmentally compatible, being

biodegradable, less toxic, and effective under different physical and chemical conditions, including stability in pH, temperature, and salinity. These properties enhance their applicability across multiple sectors, ranging from medicine, pharmaceuticals, and cosmetics to food technology, agriculture, and environmental bioremediation [1–5]. Beyond their physico-chemical advantages, biosurfactants are also recognized for their antimicrobial potential, which is of increasing interest in light of the global challenge of antimicrobial resistance.

Rhamnolipids derived from *Pseudomonas fluorescens* have been particularly highlighted for their inhibitory effects against various pathogenic bacteria [6]. In this context, investigating the antibacterial properties of biosurfactants synthesized by *Pseudomonas fluorescens* ICCF 392 against *Bacillus cereus*, a Gram-positive bacterium associated with foodborne infections, provides valuable insights into their possible role as eco-friendly alternatives to traditional antimicrobial agents.

2. Materials and Methods

2.1. Biologic Material

The microbial strain *Pseudomonas fluorescens* ICCF 392, employed in this study, is part of the Collection of Microorganisms of Industrial Importance (CMII-ICCF-WFCC 232).

2.2. Culture Media and Cultivation Conditions

The strain was maintained on M44 agar slants, with the following composition (% *w*/*v*): glycerol 5.0, yeast extract 1.0, bacto-peptone 1.0, and agar 2.0. The medium was prepared in distilled water, adjusted to pH 6.5–7.0, and sterilized for 15 min at 121 °C. Preinoculum cultures were obtained by incubating the strain at 30 °C for 48–72 h on M44 agar plates.

For biosurfactant production, the inoculum and fermentation media contained (% *v*/*v*): glycerol 3.0 and waste cooking oil 2.0 as carbon sources, yeast extract 1.0 and bacto-peptone 1.0 as nitrogen sources, and KH_2PO_4 0.2 as mineral salts. The media were adjusted to pH 7.0–7.2, sterilized at 121 °C for 15 min, and cultivated in 500 mL Erlenmeyer flasks containing 100 mL medium. Submerged fermentation was carried out at 30 °C for 72 h on a rotary shaker at 220 rpm.

2.3. Qualitative Assessment of Biosurfactant Production

The biosurfactant production by *Pseudomonas fluorescens* ICCF 392 was first assessed qualitatively. At the end of fermentation, the emulsification index ($E_{24\%}$) was determined. For this purpose, 4 mL of supernatant was mixed with 6 mL of heptane, octane, and sunflower oil in separate tubes. Then, tubes were mixed vigorously for 2–5 min and kept for 24 h [7]. The emulsification index was determined by using the following formula:

$$E_{24}\% = (\text{height of the emulsified layer/total height of the liquid column}) \times 100 \quad (1)$$

The experiments were performed in triplicate.

2.4. Isolation, Partial Purification, and Antibacterial Activity of Biosurfactant

The biosurfactant produced by *Pseudomonas fluorescens* ICCF 392 was isolated after 72 h of submerged fermentation by centrifugation, acid precipitation (pH 2.0, 2N HCl, 4 °C), and ethyl acetate extraction. The organic phase was concentrated under reduced pressure to obtain a partially purified biosurfactant. Its antibacterial activity was assessed against *Bacillus cereus* using the agar diffusion (cylinder-plate) method adapted from Niamah et al. [8]. Nutrient agar medium (g/v: meat extract 3.0, bacto-peptone 1.0, NaCl 5.0, agar 2.0) was inoculated with *B. cereus* suspension (10^8 CFU/mL), and 15 mL of medium

were poured into Petri plates to solidify. Two sterile stainless cylinders were positioned 28 mm from the plate center, and 200 μL of the partially purified biosurfactant were applied to each. Plates were incubated at 30–35 °C for 18–24 h, and inhibition zones were measured. Results are reported as the mean of three independent assays.

3. Results and Discussion

Following 72 h of submerged fermentation, the culture broth was centrifuged, and the supernatant was analyzed to assess its emulsifying properties against hydrophobic compounds. The biosurfactant exhibited pronounced emulsifying activity, consistently yielding emulsification indices above 50% for both simple hydrocarbons and vegetable oil, reflecting its effective surface-active properties. These observations suggest that the biosurfactant can effectively interact with both simple hydrocarbons and more complex lipid substrates, highlighting its versatility for industrial applications.

As illustrated in Figure 1, the antimicrobial potential of the partially purified biosurfactant was evaluated against *Bacillus cereus*, a Gram-positive pathogen often implicated in foodborne illnesses. The agar diffusion (cylinder–plate) method revealed a reproducible inhibition zone of 20 mm, confirming its antibacterial activity.

Figure 1. Antibacterial activity of biosurfactant against *Bacillus cereus* strain.

The observed inhibitory effect aligns with previous study on rhamnolipids from *Pseudomonas* species, further supporting the strain's potential as a source of natural antimicrobial agents [6]. The ability to inhibit microbial growth underscores its potential applicability across multiple sectors. In food formulations, a single biosurfactant could act simultaneously as an emulsifier and a preservative, reducing the need for additional chemical additives. In pharmaceutical applications, its surface-active and antimicrobial properties could be leveraged for drug delivery systems or surface coatings. Moreover, the use of waste cooking oil as a carbon source exemplifies a sustainable approach, aligning biosurfactant production with circular economy principles and waste valorization.

4. Conclusions

The study demonstrates that *Pseudomonas fluorescens* ICCF 392 produces a biosurfactant with significant antibacterial activity against *Bacillus cereus*. The inhibition zone of 20 mm highlights the biosurfactant's potential as a natural antimicrobial agent, particularly relevant for food safety, pharmaceuticals, and other sectors where synthetic chemicals are currently used. Utilizing inexpensive agro-industrial by-products, such as waste cooking oil, as substrates not only lowers production costs, but also contributes to sustainable bioprocessing practices.

Future work should include detailed chemical characterization, optimization of fermentation conditions for enhanced yield, and evaluation of the biosurfactant's activity against a broader spectrum of microorganisms. Additionally, testing its performance in real formulations and exploring potential synergistic effects with conventional antimicrobials could further expand its industrial relevance. Overall, the findings support the

concept that microbial biosurfactants offer a sustainable and multifunctional alternative to synthetic surfactants.

Funding: This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2021-3528, 731PED/2022, within PNCDI III.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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