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Production and Antibacterial Activity of a Biosurfactant from Schizosaccharomyces pombe Grown on Waste Soybean Oil and Corn Steep Liquor

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Abstract

Biosurfactants are microbially derived amphiphilic compounds with potential applications in environmental and industrial processes. This study evaluated biosurfactant production by the fission yeast *Schizosaccharomyces pombe* using waste soybean oil and corn steep liquor as low-cost production substrates and assessed the antibacterial activity of the recovered extract against four selected bacterial isolates. The yeast was cultivated for 120 hours at 28 °C and 150 rpm. The cell-free culture broth was acidified and extracted with ethyl acetate, followed by solvent partitioning and thin-layer chromatography (TLC). Surface activity was assessed using an oil-displacement assay and the emulsification index (E24) against vegetable, coconut, groundnut and olive oils. Antibacterial activity was evaluated by agar-well diffusion, and the minimum inhibitory concentration (MIC) was estimated by broth serial dilution. The extract produced a 29 mm oil-displacement zone and E24 values ranging from 27.8% to 56.4%. TLC with iodine vapour indicated the presence of a lipid-containing fraction; this result was considered supportive of, but not sufficient to establish, a specific phospholipid structure. The extract produced measurable inhibition at 100 and 50 mg/mL against all four isolates, while inhibition remained detectable at 25 mg/mL for *Escherichia coli*and *Staphylococcus aureus*. The corresponding MIC was therefore 25 mg/mL for these two isolates under the assay conditions used. The findings provide preliminary evidence that *S. pombe* can produce a surface-active extract when grown on low-cost waste substrates. Further work with replicated experiments, standardized antimicrobial testing, quantitative yield determination, and chemical characterization is required to confirm the composition, potency and application potential of the product.

1.0 INTRODUCTION

Surfactants occupy a central place in modern bioengineering and industrial chemistry, and their global demand has been projected to keep expanding as hygiene- and health-related applications, such as hand sanitizers and anti-inflammatory formulations, gained prominence during the COVID-19 pandemic (Subramaniam et al., 2020). Structurally, surfactants are amphiphilic molecules possessing both hydrophilic and hydrophobic domains within a single molecule, and they are broadly grouped as synthetic or microbial depending on whether they arise from chemical synthesis or microbial metabolism. Petrochemical-derived surfactants remain dominant across the pharmaceutical, agricultural, environmental-remediation and petroleum sectors, but their persistence and toxicity have raised concerns about their long-term environmental impact (Liu, 2020; Johnson et al., 2021).

Biosurfactants, the microbially synthesized counterparts of chemical surfactants, are produced by a wide range of bacteria, fungi and yeasts, although the yield and character of the molecule obtained depend heavily on the producing organism, the composition of the growth medium, the nature of the carbon substrate and the physiological conditions prevailing during cultivation (Tahir et al., 2021; Prasad *et al.*, 2021). Biosynthesis may occur intracellularly or be secreted into the medium, typically intensifying during the exponential or stationary growth phase once nutrients become limiting (Tahir et al., 2021). The ecological origin of the producing strain also shapes the properties of the biosurfactant: organisms recovered from contaminated environments frequently produce molecules tailored to metabolizing the specific pollutant present at the isolation site, presumably because the contaminant itself functions as a carbon or energy source that non-producing competitors cannot exploit (Patowary et al., 2017). Beyond their surface activity, biosurfactants contribute to cellular signalling, membrane differentiation and the mobilization of soil carbon sources (Saharan *et al.*, 2021).

Interest in biosurfactants has grown steadily because of their environmental compatibility relative to petrochemical surfactants and the possibility of producing them through fermentation from renewable feedstocks (Foley et al., 2012; Bhadani et al., 2020). In addition to biodegradability, biosurfactants offer low toxicity, functional stability across a range of pH and temperature conditions, and utility in emulsification, solubilization and the reduction of surface and interfacial tension (Van Hamme et al., 2006; Nitschke and Silva, 2018). They are typically produced by bacteria, filamentous fungi or yeasts and may take the chemical form of glycolipids, phospholipids, lipopeptides, fatty acids, saponins or alkyl polyglycosides, with hydrophilic moieties ranging from amino acids to mono-, di- and polysaccharides (Singh et al., 2019). Bacterial biosurfactants, particularly those from *Pseudomonas* and *Bacillus*, have drawn attention for antibacterial, antifungal and antiviral activity with therapeutic potential, although their pathogenic producer organisms necessitate biosafety precautions (Ines *et al.*, 2015); this has encouraged interest in non-pathogenic alternatives such as Starmerella *(Candida) bombicola*, a sophorolipid producer (De Graeve et al., 2018).

Reports of biosurfactant bioactivity against clinical pathogens continue to accumulate: Foschi et al. (2015) documented activity against Neisseria gonorrhoeae, Morais et al. (2017) against Candida albicans, and Dusane et al. (2010) described rhamnolipid-mediated biofilm degradation in *Bacillus pumilus.* Rhamnolipids appear more effective against Gram-positive than Gram-negative bacteria because the outer membrane of Gram-negative organisms can exclude the biosurfactant molecule (Bharali *et al.*, 2011), whereas lipopolysaccharide-based biosurfactants act by penetrating and destabilizing membrane lipids, producing pore formation that is particularly damaging to Gram-negative pathogens (Naughton et al., 2019). Beyond antibacterial use, biosurfactants have shown anticancer activity by disrupting membrane integrity and promoting apoptosis (Gudina et al., 2013; Zhao et al., 2018; Dey et al., 2015), and are increasingly incorporated into drug-delivery systems, wound-healing hydrogels and cosmetic formulations because of their favourable toxicity and dermal compatibility profiles (Sandeep et al., 2017; Ferreira et al., 2017; Adu et al., 2020).

The cost of biosurfactant production remains a major barrier to industrial adoption, with raw materials accounting for 10–30% of total production cost and downstream recovery and purification consuming up to 60% (Mukherjee et al., 2006; McInerney et al., 2005). This has driven interest in inexpensive, renewable substrates such as frying oil, distillery waste, molasses and plant-derived oils as replacements for refined glucose or sucrose (Patel et al., 2021; Almeida et al., 2004; De Lima et al., 2009). Vegetable oils and other hydrocarbon-based residues have proved particularly economical for large-scale biosurfactant production from *Pseudomonas, Bacillus* and *Candida* species (Sivapathasekaran and Sen, 2017). Comparative substrate trials illustrate this variability: Agarwal and Sharma (2009) found glycerol and molasses gave similar biosurfactant activity to glucose, while ammonium chloride, ammonium nitrate and sodium nitrate all supported comparable yields as nitrogen sources. Al-Bahry et al. (2013) recovered 2.29 g/L of biosurfactant from Bacillus subtilis grown on date molasses, reducing surface tension from 60 to 25 mN/m, while Hentati et al. (2019) obtained 50 mg/L of biosurfactant from Bacillus stratosphericus using residual frying oil, lowering surface tension from 72 to 28 mN/m. These reports collectively support the rationale for using waste soybean oil and corn steep liquor, both inexpensive and widely available by-products, as the production substrates in the present study.

Biosurfactants also have an expanding footprint in pharmaceutical and cosmetic applications, owing to antimicrobial, anti-adhesive, antiviral, anticancer, haemolytic, anti-inflammatory and immunomodulatory activities documented across multiple producer organisms (Drakontis and Amin, 2020; Gomaa, 2013). Surfactin, for instance, exhibits antimicrobial, antiviral, antitumour, hypocholesterolemic, anti-adhesive and apoptotic properties, and inhibits fibrin clotting alongside antitumour activity against Ehrlich's ascites carcinoma cells (Arima et al., 1968; Gudina et al., 2013). In cosmetic formulation, biosurfactants offer lower toxicity and better dermal compatibility than conventional surfactants such as sodium dodecyl sulphate (Sandeep et al., 2017; Ferreira et al., 2017), and several commercial products, including the moisturizer Kanebo, the facial cleanser Sopholiance and the body moisturizer Relipidium, already incorporate microbial biosurfactants (Adu et al., 2020). These applications reinforce the rationale for characterizing new, low-cost biosurfactant sources such as *S. pombe*.

The rising tide of antimicrobial resistance, driven by Inappropriate drug use, poor prescription compliance and the acquisition of resistance genes among microbial populations, has intensified the search for alternative antimicrobial strategies. Because biosurfactants are amphiphilic compounds capable of interacting with phase boundaries in heterogeneous biological systems, several reports have highlighted their activity against otherwise resistant organisms, positioning them as candidate antimicrobial agents worth further evaluation.

Despite this growing body of evidence, the biosurfactant-producing capacity of *S. pombe* and the antibacterial performance of the resulting extract remain comparatively under-characterized. The present study therefore aimed to evaluate biosurfactant production by a pure isolate of *S. pombe* cultivated on low-cost renewable substrates and to assess the antibacterial activity of the resulting extract against four selected bacterial isolates.

2.0 MATERIALS AND METHODS

All chemicals and solvents used, including ethyl acetate, dichloromethane, hydrochloric acid, sodium chloride, magnesium sulphate and chromatographic visualization reagents, were of analytical grade. Nutrient agar and nutrient broth were used for maintenance and antibacterial testing. Waste soybean oil and corn steep liquor served as the carbon and nitrogen sources, respectively, for yeast fermentation. Ciprofloxacin was used as the reference antibacterial agent in the comparative agar-well diffusion assays.

2.1 Source of Organisms

Pure stock cultures of the fission yeast *S. pombe* and four selected bacterial isolates (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Shigella dysenteriae*) were obtained from the culture collection of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. The yeast culture was transferred to the mycology laboratory of the same department for analysis, while the bacterial isolates were maintained at 4°C in the microbiology laboratory pending further use. The available manuscript information does not provide independent clinical provenance or antimicrobial-resistance profiles for the isolates; therefore, they are referred to here as selected bacterial isolates.

2.2 Biosurfactant Production

A 2% (v/v) inoculum of *S. pombe* (approximately 10^8 cells/mL) was introduced into a production medium containing 1.0% (w/v) waste soybean oil and 1.0% (w/v) corn steep liquor in distilled water, adjusted to pH 6.8. Cultures were incubated at 28°C with agitation at 150 rpm for 120 hours (Selvakumar et al., 2016). This single cultivation condition was used as the production condition examined in the present study; no factorial or response-surface optimization experiment was performed.

2.3 Biomass Determination

Biomass was quantified by centrifuging 100 mL of culture broth in pre-weighed tubes at 12,000 × g for 20 minutes. The resulting pellet was washed twice with distilled water and dried in a hot-air oven at 105°C for 24 hours before the dry weight was recorded (García-Ochoa, 1999).

2.4 Biosurfactant Extraction

Extraction followed the method of Luna et al. (2012) with minor modification. Cell-free culture broth was acidified to pH 2.0 with 6 N HCl and held overnight at 4°C. The acidified broth was then extracted three times with an equal volume of ethyl acetate. The pooled organic phases were dried after removal of residual water using saturated sodium chloride and anhydrous magnesium sulphate, and the solvent was removed at 40°C under reduced pressure. The recovered material was used for subsequent partial purification and activity assays.

2.5 Purification of the Biosurfactant

The dried extract was re-suspended in 40 mL of dichloromethane in a separating funnel and shaken vigorously to partition the extract into the organic phase; this step was performed twice, and the pooled organic phases were evaporated to dryness. Partial purification was assessed by thin-layer chromatography (TLC) on silica plates developed in a chloroform/methanol/water solvent system (Caldini et al., 1995). Ninhydrin reagent and iodine vapour were used to visualize protein- and lipid-containing fractions, respectively. Iodine-positive TLC staining was interpreted only as evidence of a lipid-containing fraction; it was not used alone to establish a phospholipid structure or molecular identity.

2.6 Oil-Displacement Assay

Thirty millilitres of distilled water were placed in a Petri dish and 1 mL of crude oil was layered onto the centre of the water surface. Twenty microlitres of culture supernatant were then dropped onto the centre of the oil film. A positive result was recorded when the biosurfactant displaced the oil and formed a visible clearing zone in the water (Hsieh et al., 2004; Youssef et al., 2004; Thavasi et al., 2011; Ben Belgacem et al., 2015).

2.7 Emulsification Index (E24)

Emulsifying capacity was determined for four hydrophobic substrates (vegetable oil, coconut oil, groundnut oil and olive oil). For each substrate, 2 mL of the oil was combined with 2 mL of cell-free broth in a test tube, vortexed vigorously for 2 minutes, and left undisturbed for 24 hours. The emulsification index (E24) was calculated as the height of the stable emulsion layer divided by the total height of the liquid column, multiplied by 100 (Bodour et al., 2004).

2.8 Antibacterial Activity

Three biosurfactant concentrations (25, 50 and 100 mg/mL) were prepared by dissolving 0.1, 0.2 and 0.4 g of recovered extract, respectively, in 4 mL of distilled water. Antibacterial activity was assessed by the agar-well diffusion method based on the Kirby-Bauer approach (1998). Nutrient agar plates were inoculated with 20 µL of a 24-hour broth culture of the relevant test isolate. Wells of 6 mm diameter were bored into the agar and received biosurfactant extract, ciprofloxacin, or distilled water as the controls. Zones of inhibition were measured in millimetres. The original study report does not provide sufficient replicate-level information, inoculum standardization details, or the exact ciprofloxacin concentration used; therefore, the inhibition-zone data are presented as descriptive observations and should not be interpreted as a standardized potency comparison.

2.9 Minimum Inhibitory Concentration (MIC)

The MIC of the biosurfactant extract was estimated by broth serial dilution. Ten millilitres of nutrient broth were dispensed into each of five test tubes, and 0.2 mL of a 24-hour broth culture of the relevant test isolate was added to each tube. A 100 mg/mL biosurfactant stock was serially diluted in nutrient broth by transferring 5 mL between successive tubes to yield final concentrations of 100, 50, 25, 12.5 and 6.25 mg/mL. The tubes were incubated at 37°C for 24 hours before turbidity was assessed (Andrews, 2004). Because the original report does not document standardized inoculum density, biological replicates, or solvent and sterility controls, the MIC findings should be regarded as preliminary and specific to the assay conditions used.

2.10 Data Presentation and Analysis

The study outcomes are presented descriptively because replicate-level measurements and inferential statistical test results were not reported in the available study record. Numerical observations are therefore reported as recorded, without introducing unverified estimates of variability or statistical significance.

3.0 RESULTS

3.1 Oil-Displacement Activity

The crude extract from *S. pombe* produced a clear oil-displacement zone measuring 29 mm in diameter, indicating surface-active activity in the culture supernatant under the assay conditions used.

3.2 Emulsification Index

The emulsifying capacity of the biosurfactant varied with the hydrophobic substrate used (Table 1). Vegetable oil gave the highest emulsification index (56.4%), followed by coconut oil (51.6%) and olive oil (44.6%), while groundnut oil gave the lowest value (27.8%).

Table 1
Emulsification index (E24) of the biosurfactant against different hydrophobic substrates.

Hydrophobic Substrate	Emulsification Index (%)
Vegetable oil	56.4
Coconut oil	51.6
Groundnut oil	27.8
Olive oil	44.6

3.3 Thin-Layer Chromatography

Thin-layer chromatography of the partially purified extract produced a brownish spot after exposure to iodine vapour, indicating the presence of a lipid-containing fraction (Fig. 2). TLC with iodine vapour alone does not establish a specific phospholipid class or molecular structure; confirmation would require additional chemical characterization.

3.4 Antibacterial Activity

All four test isolates showed measurable inhibition with the biosurfactant at 100 and 50 mg/mL (Table 2). At 25 mg/mL, inhibition remained detectable for *Escherichia coli* and *Staphylococcus aureus*, whereas *Salmonella typhi* and *Shigella dysenteriae* showed no measurable inhibition at this concentration or below. No activity was recorded for any isolate at 12.5 or 6.25 mg/mL. On the basis of the dilution series, the MIC was estimated at 25 mg/mL for *E. coli* and *S. aureus* under the assay conditions used.

Table 2
Antibacterial activity of the *S. pombe* biosurfactant at decreasing concentrations.

Test Isolate	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL
Salmonella typhi	+	+	-	-	-
Escherichia coli	+	+	+	-	-
Staphylococcus aureus	+	+	+	-	-
Shigella dysenteriae	+	+	-	-	-

Key

+ = activity observed (zone of inhibition present); − = no activity observed.

3.5 Comparison with Ciprofloxacin

Zones of inhibition produced by the biosurfactant were descriptively compared with those reported for ciprofloxacin at the tested assay concentrations (Tables 3–5). At 100 mg/mL, the biosurfactant produced larger inhibition zones than ciprofloxacin against *E. coli* (8 mm versus 5 mm) and *S. dysenteriae* (5 mm versus 2 mm), whereas ciprofloxacin produced larger zones against *S. typhi* (9 mm versus 5 mm) and *S. aureus* (7 mm versus 6 mm). At 50 mg/mL, the biosurfactant again produced larger recorded zones against *E. coli* (6 mm versus 2 mm) and *S. dysenteriae* (2 mm versus 1 mm), while ciprofloxacin produced larger zones against *S. typhi* and *S. aureus*. At 25 mg/mL, the biosurfactant showed inhibition against *E. coli* and *S. aureus* (2 mm each), whereas the recorded ciprofloxacin zones were larger for the isolates that remained inhibited. These differences should be interpreted as assay-specific inhibition-zone observations rather than evidence that the biosurfactant is more potent than ciprofloxacin, because the study does not report standardized or directly equivalent test concentrations and replicate variability.

Table 3
Zone of inhibition (mm) produced by distilled water, ciprofloxacin and the biosurfactant at 100 mg/mL.

Test Isolate	Distilled Water (mm)	Ciprofloxacin (mm)	Biosurfactant (mm)
Salmonella typhi	0	9	5
Escherichia coli	0	5	8
Staphylococcus aureus	0	7	6
Shigella dysenteriae	0	2	5

Table 4
Zone of inhibition (mm) at 50 mg/mL of biosurfactant and ciprofloxacin.

Test Isolate	Distilled Water (mm)	Ciprofloxacin (mm)	Biosurfactant (mm)
Salmonella typhi	0	5	3
Escherichia coli	0	2	6
Staphylococcus aureus	0	4	3
Shigella dysenteriae	0	1	2

Table 5
Zone of inhibition (mm) at 25 mg/mL of biosurfactant and ciprofloxacin.

Test Isolate	Distilled Water (mm)	Ciprofloxacin (mm)	Biosurfactant (mm)
Salmonella typhi	0	5	0
Escherichia coli	0	2	2
Staphylococcus aureus	0	3	2
Shigella dysenteriae	0	0	0

4.0 DISCUSSION

The 29 mm zone of oil displacement obtained in this study indicates that *S. pombe* produced surface-active compounds capable of disturbing an oil/water interface under the assay conditions used. The variation in emulsification index across the tested substrates, from 27.8% with groundnut oil to 56.4% with vegetable oil, is consistent with substrate-dependent differences in emulsion formation. These results support the presence of surface-active material but do not by themselves establish product purity or chemical identity.

The iodine-positive TLC signal indicates that the recovered extract contains lipid material, but it does not by itself establish whether the active molecule is a phospholipid, glycolipid or another lipid-containing compound. The present finding is therefore best interpreted as preliminary compositional evidence. Structural identification of the active fraction would require additional analytical approaches such as FTIR, mass spectrometry, NMR, phosphorus-specific analysis, or comparison with appropriate authentic standards.

The antibacterial results show a concentration-dependent pattern within the tested range. At 100 and 50 mg/mL, the biosurfactant produced measurable inhibition against all four isolates, while at 25 mg/mL inhibition remained detectable only for *E. coli* and *S. aureus.* The inhibition zones recorded for the biosurfactant were sometimes larger than those recorded for ciprofloxacin under the particular assay conditions, but these observations should not be interpreted as evidence of superior antimicrobial potency because the study does not provide standardized antibiotic concentrations, replicate-level variability, or a formal statistical comparison.

Differences in susceptibility among the four isolates may reflect differences in cell-envelope structure and other organism-specific properties. However, the present study did not investigate the mechanism of action of the extract. Mechanistic explanations involving membrane disruption or selective activity against Gram-negative organisms therefore remain hypotheses that require direct experimental testing.

The overall findings support the conclusion that *S. pombe* cultivated on waste soybean oil and corn steep liquor can generate a surface-active extract with measurable antibacterial activity under the tested laboratory conditions. The results are encouraging but preliminary, particularly because the extract was only partially purified and the chemical identity of the active component was not established.

The relevance of these findings to antimicrobial resistance should also be interpreted cautiously. The study did not characterize the resistance phenotypes or genotypes of the bacterial isolates, nor did it determine the mechanism by which the biosurfactant inhibited growth. Consequently, the present data do not demonstrate that the extract overcomes antibiotic resistance or that it is less susceptible to established resistance mechanisms. Future work should investigate mechanism of action and potential interactions with conventional antibiotics using standardized experimental designs.

5.0 LIMITATIONS

Several limitations affect interpretation of the present findings. First, only a single fermentation condition (28°C, 150 rpm, 120 h, pH 6.8) was examined, so the study demonstrates production under a selected condition rather than optimization of the production process. Second, the antibacterial extract was only partially purified. Third, the available study record does not provide replicate-level measurements, standardized inoculum density, detailed control conditions, or statistical analysis. Finally, TLC with iodine staining is insufficient for definitive chemical

classification. Future studies should therefore incorporate factorial or response-surface optimization of substrate ratio and process variables, independent biological replicates, standardized antimicrobial susceptibility methods, and comprehensive structural characterization.

5.0 CONCLUSION AND RECOMMENDATIONS

This study provides preliminary evidence that the fission yeast *S. pombe*, grown on a medium containing waste soybean oil and corn steep liquor, can produce a surface-active extract with measurable antibacterial activity. The extract produced a 29 mm oil-displacement zone and E24 values ranging from 27.8% to 56.4% across the tested oils. In the antibacterial assays, inhibition was observed at 100 and 50 mg/mL for all four isolates and remained detectable at 25 mg/mL for *E. coli* and *S. aureus*. The present experiments support biosurfactant activity but do not establish a definitive phospholipid structure, optimized production conditions, or clinical/therapeutic efficacy.

Further studies should focus on true process optimization using replicated experimental designs, quantitative biosurfactant-yield measurement, standardized antimicrobial susceptibility and MIC testing, and detailed chemical characterization of the active fraction. No toxicity or dermal-compatibility testing was performed in this study, so medical, pharmaceutical and cosmetic applications remain hypothetical until safety and efficacy are established experimentally.

From an industrial perspective, the use of waste soybean oil and corn steep liquor provides a rational low-cost substrate strategy and may contribute to waste valorization. However, industrial feasibility cannot be established from the present study because fermentation yield, recovery efficiency, process economics, product consistency and scale-up performance were not comprehensively evaluated. These factors should be investigated before conclusions about commercial application are made.

S. pombe is a well-characterized laboratory yeast with an established genetic toolkit, which may make it attractive for future strain and pathway engineering. In the context of this study, however, this represents a future development opportunity rather than an experimentally demonstrated advantage for biosurfactant production. Subsequent work could investigate the metabolic pathways underlying production and assess whether strain or process engineering improves yield and antibacterial activity.

Declarations

Author Contribution

Author contributions:Study concept and design: Abdullahi Usman Ndabata, and Muhammad LimanData collection: Abdullahi Usman Ndabata, Zephaniah Isaiah, and Aisha IbrahimDrafting and revision of paper: Belema Joshua Okoseimiema, Abba Stephen Paul, and Samson Tesfaye GebreAll authors have read, edited, and contributed to the content of this manuscript. This work has not been previously published and has not been considered for publication elsewhere.

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Figures

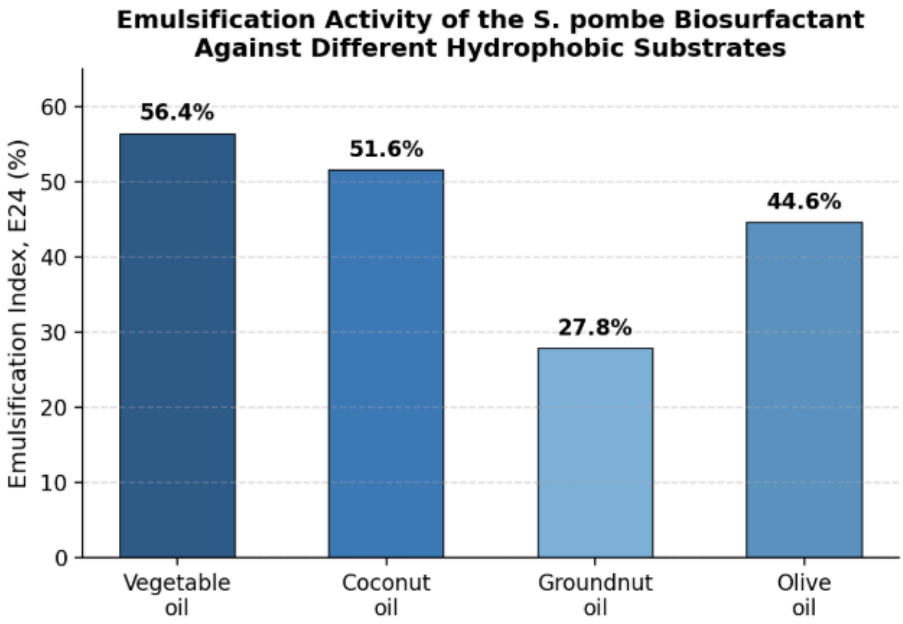


Figure 1

Emulsification index (E24) of the *S. pombe* biosurfactant against four hydrophobic substrates.



Figure 2

Thin-layer chromatogram of the partially purified *S. pombe* biosurfactant after iodine vapour staining; the brownish spot indicates a lipid-containing fraction

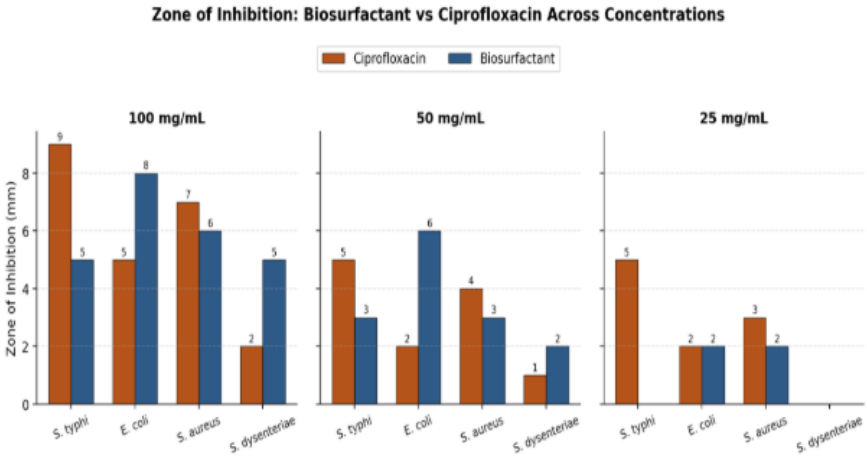


Figure 3

Zone of inhibition (mm) produced by the biosurfactant versus ciprofloxacin against the four test isolates at 100, 50 and 25 mg/mL.