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# Isolation, Characterization and Antimicrobial activity of Biosurfactant Produced by Yeast isolate YNWaG

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**A B S T R A C T**

Biosurfactants are extracellular secondary metabolites produced by microorganisms such as bacteria, yeast and fungi. These biological surface-active compounds exhibit potential antimicrobial activity. The present study aimed to isolate the biosurfactant producing yeast from fruits, vegetables and edible flowers (roses), and to evaluate the antibacterial activity. A total twenty yeast isolates were obtained and screened for biosurfactant production using oil displacement method and emulsification index ($E_{24}$). Among them, YNWaG exhibited the highest activity with oil displacement diameter of 5.16±0.05cm and emulsification index ($E_{24}$%) of 51.13±0.15. The biosurfactant produced by the selected yeast isolate YNWaG was further assessed for antibacterial activity against *E. coli* and *S. aureus* by agar well diffusion method. It was observed that the zone of inhibition increased with an increase in the concentration of biosurfactant. The zone of inhibition of *E. coli* increased from 27. 9±0.55 to 34.7±0.73 and for *S. aureus* from 16.1±0.45 to 31.5±0.50 with an increase in biosurfactant concentration from 1.25 mg/mL to 2.5 mg/mL. Statistical analysis using Student's t-test indicates that the difference is highly significant ($p < 0.001$). Yeast derived biosurfactants are generally regarded as safe. The findings of this study shows that the yeast-derived biosurfactant can be used in healthcare, cosmetic industries and agriculture as nontoxic antimicrobial agents.

## Introduction

Chemical surfactants are synthesized from petroleum feedstocks, it causes various environmental risks due to their non-degradability and toxicity to aquatic and terrestrial ecosystems (Nunes RF, Teixeira, 2022). Biosurfactants are the amphiphilic biomolecules synthesized by the microorganisms. These naturally derived surfactants have gained attention due to the desirable characteristic properties such as biodegradability, reducing surface tension, less toxicity and stability under adverse conditions (I.G.S Da Silva, *et al.,* 2020). In addition, biosurfactant can be produced from renewable substrates and thus providing economic

benefits. As a result, it led to the growing interest of the scientific studies on a wide range application of biosurfactants in various fields such as agriculture, bioremediation, food processing, health, cosmetic and pharmaceutical industries (Boxley CJ, *et al.,* 2023).

Biosurfactant possess significant properties including high foaming capacity, emulsification, and wetting properties due to which they have gained importance over conventional chemical surfactants (Shaimerdenova U, *et al.,* 2024). Biosurfactant produced by yeast is gaining much importance because yeast is usually considered as non-pathogenic and hence the yeast derived biosurfactants are generally non-toxic and biodegradable. Biosurfactants also exhibits significant antimicrobial properties with therapeutic and biomedical potential (Inès M., Dhouha G 2015). These properties make them ideal for its application in food, cosmetic and pharmaceutical industries. Biosurfactant producing yeast mainly includes *Saccharomyces sp*., *Pichia sp.*, *Candida sp.*, *Yarrowia sp.* (Barth G, Gaillard C., 1997).

In this study we focussed on the isolation of potential biosurfactant producing yeast from various fruits and vegetables and to evaluate the antimicrobial activity of the yeast derived biosurfactant against selected gram positive and gram-negative pathogens.

## Materials and Methods

### Isolation of yeast from different fruit and vegetable samples

For the isolation of yeast from fruit samples (Pear, Dragon fruit, Blue Berries, Cherries, Dates, Wood apple, Plums), vegetables (Ginger, Sweet potato) and edible flowers (Roses), 10 gm of different samples were mixed in 20 mL of sterilized water in separate conical flask and incubated for about 48 hr at room temperature. Enrichment culturing technique were used for the processing of the samples for which 10 mL of the fermented sample were inoculated into 100 mL of YEPD broth (1% yeast extract w/v, 2% peptone w/v, 2% dextrose w/v) and incubated on rotary shaker at 200 rpm for 24 hr. The enriched samples were then serially diluted and then plated on YEPD agar medium; plates were then incubated at 30 $^{O}$C for 48 hr. Isolated colonies were obtained, their morphological characteristics were studied under the microscope and maintained as pure culture.

### Screening for biosurfactant producing yeast

### Oil displacement assay

The ability of the yeast isolates to produce biosurfactant were assessed using oil displacement assay. This assay was performed in a 100 mm diameter petri dish containing 20 mL distilled water that was overlaid with 20 µL of engine oil. To this, add 10 µL of cell free supernatant onto the centre of the oil layer (Mulligan *et al.,* 1984). Appearance of halo surrounded by engine oil is considered positive for biosurfactant production. SDS was used as a positive control and phosphate buffer as negative control.

### Emulsification assay (E24%)

The emulsification index ($E_{24}$ %) of cell free supernatant were assessed as described by Cooper and Goldenberg (1987). Equal volumes of soyabean oil and cell free supernatant were taken in test tubes and vortexed for 2 min and left undisturbed for 24 hr. The measurements were taken after 24 hr and the emulsification index was calculated as below:

$$E24\ \% = \frac{\text{Height of emulsified layer}}{\text{Height of total mixture}} \times 100$$

### Biosurfactant production from YNWaG

Single isolated colony of YNWaG is inoculated into 100 mL YEPD medium and incubated at 30 $^{O}$C on rotary shaker at 200 rpm for 24 hr. Further,4% (v/v) of the overnight culture is inoculated in the mineral salt medium (MSM) of the composition (w/v) 0.2% $KH_2PO_4$, 0.2% $MgSO_4$, 0.2% KCl, 0.2% $Na_2CO_3$, supplemented with 2% glucose, 2% (v/v) glycerol and 0.02% chloramphenicol and incubated at 30 $^{O}$C on rotary shaker at 150 rpm for 96 hr (Daniel Loeto *et al.,* 2021)

### Liquid-Liquid extraction of Biosurfactants

The yeast culture grown on MSM was centrifuged at 10,000 rpm, 4 $^{O}$C for 12 min to obtain a cell free supernatant. The biosurfactant is extracted from the supernatant by liquid-liquid extraction method using ethyl acetate solvent. Equal volumes of supernatant and ethyl acetate were taken in separating flask and the organic phase is separated. The separated organic phase

is vacuum dried using rotary evaporator. The concentrated biosurfactant obtained is washed with n-hexane to remove the residues

## Antibacterial activity of biosurfactants

The antimicrobial activity of biosurfactant was evaluated using agar well diffusion method. Sterile Nutrient agar plates were inoculated with 100µL of bacterial culture of *E,coli* and *S.aureus* by evenly spreading the inoculum. Aseptically punch the wells of 6mm diameter on to the inoculated plates. Load the wells with 50µL and 100µL of the extracted biosurfactant. Allow the plates to stand at room temperature for 30 min. to facilitate the diffusion. Incubate the plates at 37 $^{O}$C for 24 hrs. The antimicrobial activity is determined by measuring the diameter of zone of inhibition (De Giani A, *et al.,* 2021). The experiment is performed in triplicates.

## Statistical analysis

The results are expressed as mean ± standard deviation. Statistical analysis was performed using an unpaired Student's t- test in Microsoft Excel. A $p < 0.05$ were considered statistically significant.

## Results and Discussion

## Isolation and screening of biosurfactant producing yeast

About twenty yeasts with distinct morphology were isolated from the collected fruits, vegetables and edible flower(roses) samples using YEPD medium. In this study only three yeast isolates labelled as YNWaG, YNRoG, YNSPO respectively which were obtained from wood apple, rose flower and sweet potato showed potential oil displacement and emulsification activity. Figure 1 shows the microscopic observation of the yeast cells. Colony characteristics such as the colour, margin and elevation were recorded (Table 1). Yeast isolate YNWaG showed maximum oil displacement and the diameter of the zone was measured to be 5.16±0.05 cm followed by isolates YNSPO and YNRoG with their zone diameters measured to be 4.53±0.05cm, and 4.4±0.1cm respectively (Table 2, Fig.2). The emulsification activity of the selected four isolates was measured. YNWaG isolate showed the highest $E_{24}$% of 51.13±0.15% whereas isolate YNRoG showed the lowest $E_{24}$% of 46.66±0.11% (Fig.3). Hence YNWaG isolate is used for subsequent studies.

## Antibacterial activity of biosurfactants

Yeast derived biosurfactants has shown antibacterial activity against *E. coli* and *S. aureus* (Fig.4). The zone of inhibition increases with concentration, maximum zone of inhibition was observed at biosurfactant concentration of 2.5 mg ml$^{-1}$ wherein, it was approximately 34.7±0.73 mm for *E. coli* and 31.5±0.50 mm for *S. aureus* (Table.3). At a concentration of 1.25 mg ml$^{-1}$ the zone of inhibition for *E. coli* is 27.9±0.55 mm and for *S. aureus* it is 16.1±0.45 mm respectively. The diameter of zone of inhibition was found to be more for *E. coli* than *S. aureus* (Fig.5).

Statistical analysis was performed using Student's t-test in Microsoft Excel. Increase in zone of inhibition with increase in biosurfactant concentration was found to be statistically significant for both the test organisms ($p < 0.01$ for *E. coli* and $p < 0.001$ for *S. aureus*). It was observed that *E. coli* showed high susceptibility even at low concentration whereas *S. aureus* showed remarkedly high increase in the diameter of zone of inhibition at a higher concentration. This difference in response is mainly due to the cell wall composition of gram positive and gram negative bacteria.

The unique amphiphilic component present in the biosurfactants is capable of interacting with the lipid component of the microorganisms altering their physico-chemical properties (Bjerk, T. R., *et al.,* 2021). This mechanism explains the antibacterial activity observed in the present study. Biosurfactants exhibit broader spectrum of antimicrobial, anti-adhesion and antibiofilm properties. Moreover, Yeast is generally recognised as safe, thus the biosurfactant produced by yeast are used in biomedical, cosmetics and personal care applications (Lourith, N. and Kanlayavattanakul, M. 2009).

In conclusion, the present study demonstrated that yeast derived biosurfactant exhibit significant, concentration dependent antimicrobial activity against the tested bacteria. Despite the promising results, the study is limited to a small number of concentrations and test organisms. Future studies should include the characterisation of biosurfactant, optimization for enhanced productivity, and evaluation of broader spectrum of antimicrobial activity of the yeast derived biosurfactant.

**Table.1** Colony characteristics of yeast isolates

| Isolates | Colony colour | Margin | Elevation | Texture/Surface |
|---|---|---|---|---|
| **YNWaG** | Cream | Smooth | Raised | Smooth |
| **YNRoG** | White | Lobate | Raised | Mucoid |
| **YNSPO** | Cream | Smooth | Flat | Smooth |

**Table.2** Screening of biosurfactant producing yeast

| Isolates | Oil Displacement Test (cm) | $E_{24}$% |
|---|---|---|
| **YNWaG** | 5.16±0.05 | 51.13±0.15 |
| **YNRoG** | 4.4±0.1 | 46.66±0.11 |
| **YNSPO** | 4.53±0.05 | 47.66±0.11 |

Data is given as mean ± STDV (n=3)

**Table.3** Effect of biosurfactants on *E. coli* and *S. aureus*

| Concentration of biosurfactant (mg $ml^{-1}$) | Zone of inhibition (mm) | |
|---|---|---|
| | ***E. coli*** | ***S. aureus*** |
| **1.25** | 27.9±0.55 | 16.1±0.45 |
| **2.50** | 34.7±0.73 | 31.5±0.50 |

Data is given as mean ± STDV (n=3)

**Fig.1** Pure culture of Yeast sp. and microscopic image under 100X

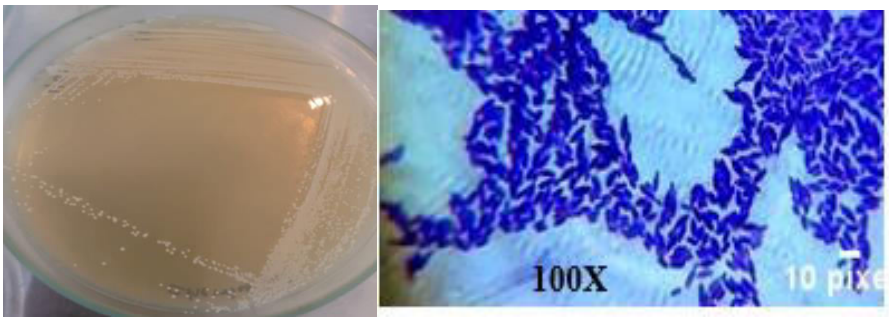



**Fig.2** Oil Displacement test of yeast isolates. (1) YNWaG (2) YNRoG (3) YNSPO

**Fig.3** Emulsification activity of different yeast strains

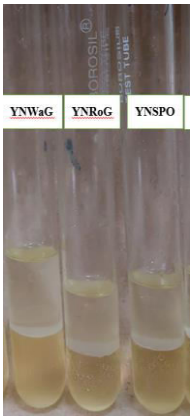



**Fig.4** Nutrient agar plates showing Zone of inhibition

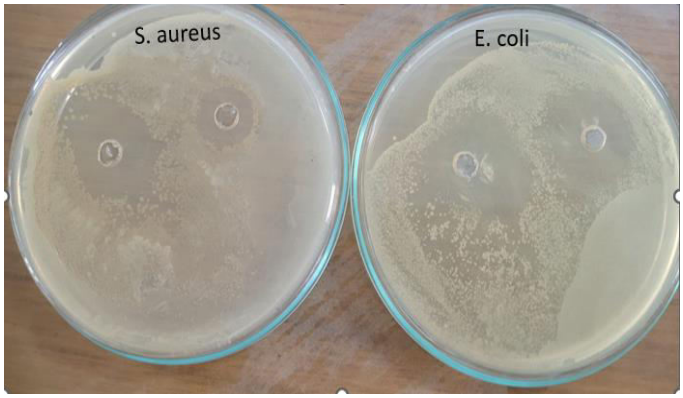
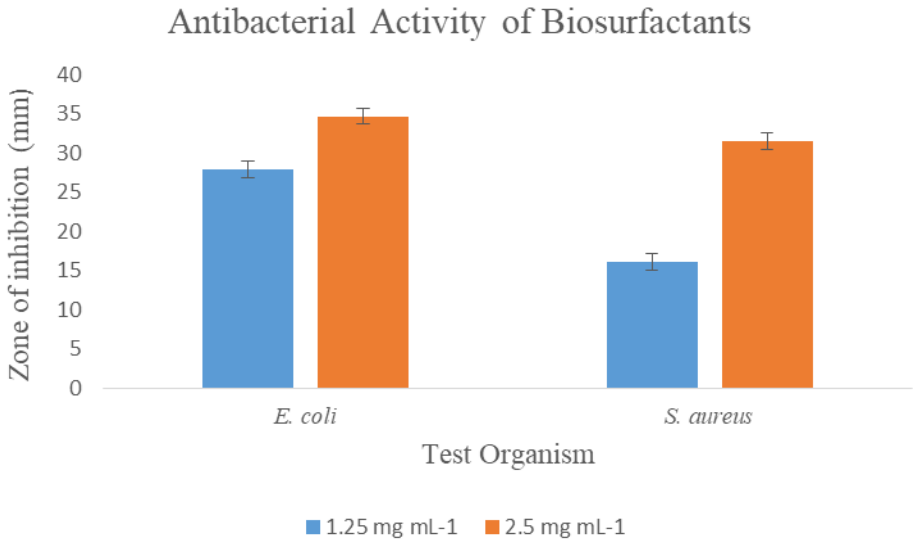



**Fig.5** Antibacterial activity of biosurfactants against *E. coli* and *S. aureus*



Hence, these findings suggest that the yeast derived biosurfactants can be a sustainable alternative to synthetic surfactants.

## Author Contributions

Nahida Fatima: Conceived the original idea and designed the model and wrote the manuscript. Yahya Khan M.: Designed the model and the computational framework and analysed the data. Anuradha B. S.: Designed the model and the computational framework and analysed the data.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare that there is no conflict of interest regarding the publication of this paper

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