RESEARCH ARTICLE

In vitro antifungal activity of the bacterial Biosurfactant

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ABSTRACT

Research in the area of biosurfactant has expanded quite a lot in recent years. Biosurfactants are amphiphilic compounds i.e., they contain both hydrophilic and hydrophobic moieties. Their low toxicity and eco-friendly nature and the wide range of potential industrial applications in health care, bioremediation, and food processing makes them a highly useful compound. They are produced by a variety of microorganisms as extracellular compounds. Biosurfactants are superior to the chemical surfactants with respect to their biocompatibility, lower toxicity, and higher biodegradability. The present study was focused on in vitro antifungal activity of rhamnolipid biosurfactant produced by bacteria *Pseudomonas aeruginosa* using olive oil as substrate. Biosurfactant was extracted by solvent extraction method using equal volume of Chloroform and n-butanol (65:15). The extracted biosurfactant was evaluated for its potential antifungal activity by using agar cup method against yeast *Candida albicans* and was compared with commercial chemical surfactant Sodium lauryl Sulphate. Extracted rhamnolipid Biosurfactant showed antifungal activity against *Candida albicans*. Thus, the work suggests that after further purification it could be used as therapeutic agent in Biomedical and pharmaceutical applications.

Key Words: Rhamnolipid Biosurfactant, Antifungal activity, olive oil, therapeutic agent.

INTRODUCTION

It is well known that the emergence of fungal infections is most common. Even though there are over 200 kinds of antibiotics, the problem of resistance to antibiotics is observed (Ahimou et al., 2000). Hence finding new therapeutic agents becomes important. Biosurfactant is a highly useful compound that significantly works as antifungal agents (Banat et al., 2010).

In the recent past, attentions have been paid to alternative, environmental friendly, surface active products synthesized by microorganisms known as biosurfactants. Biosurfactants are amphiphilic molecules mainly produced by microorganisms as a secondary metabolite. They possess both hydrophilic and hydrophobic moieties and are able to display a variety of surface activities and help to solubilize hydrophobic substrates Bacteria are the main group of biosurfactant-producing microorganisms, although it is also produced by some yeasts and filamentous fungi (Bodour et al., 2004; Desai and Banat, 1997). *Pseudomonas* species is well known for its ability to produce rhamnolipid biosurfactants with Potential surface active properties when grown on different carbon substrates and therefore is a promising candidate for large scale production of biosurfactants (Karsa et al., 1999). The present study is aimed at producing biosurfactant using olive oil as substrate by bacteria *Pseudomonas aeruginosa* and investigating it's in vitro antifungal activity against yeast *Candida albicans.*

MATERIAL AND MATERIALS

Organism

Pseudomonas aeruginosa is used in the present study for Biosurfactant production and was maintained on nutrient agar slant.

Confirmation of biosurfactant production by *Pseudomonas aeruginosa*

***Media and cultivation conditions*:**
After 24 hrs of incubation the microbial growth from slant was scraped out and suspended into 50ml of Sterile Luria Bertani broth in a 250ml Erlenmeyer flask. The flask was incubated on a rotary shaker for 4 days at 28^0C. After incubation, the content of the flask was centrifuged at 7000 rpm for 20min.Taking supernatant following tests for identifying the presence of biosurfactant were performed:

Phenol-Sulphuric Acid Method:
In 1ml of cell free supernatant 1ml of 5% phenol was added. To this mixture, 2-5ml of concentrated sulphuric acid was added drop by drop, until characteristic color was developed. Development of orange color indicated the presence of glycolipids.

***Blue agar plate method (BAP*):**
Basal Mineral salt agar medium was supplemented with (2%) glucose as carbon source and cetyltrimethyl- ammonium bromide (CTAB: 0.5 mg/mL) and methylene blue (MB: 0.2 mg/mL) for the detection of anionic biosurfactant. Wells were punched at the centre of the blue agar plate with alcohol sterilized cork borer to which 30µl of cell free supernantant was added. The plates were incubated at 37°C for 48-72 h. A dark blue halo zone around the well is considered positive for anionic biosurfactant.

***Emulsification test (E24)*:**
Several colonies of pure culture were suspended in test tubes containing 2 mL of mineral salt medium, after 48 h of incubation, 2 mL hydrocarbon (oil) was added to tube. Then, the mixture was vortexed at high speed for 1 min and allowed to stand for 24 h. The emulsification index (E24) is the height of the emulsion layer (mm) divided by total height (mm), multiplied by 100 (Bodour *et al.,* 2004).

Production and extraction of biosurfactant

***Preculture*:**
loop full microbial growth from the 24 hrs old slant was scraped out and inoculated into 100ml of Sterile Luria Bertani broth in a 250ml Erlenmeyer flask and incubated at 28^0c for 24hrs on shaker.

***Production*:**
Five different flasks containing 100ml of Basal Mineral Salt medium containing 2% Oilve oil as carbon source whereas 0.1% of Ammonium Nitrate as Nitrogen source was inoculated with

5ml of preculture and incubated at 28^{0}c for 5days on shaker.

***Extraction*:**

Acid precipitation**:** Remove Cells from the culture broth by centrifugation at 10 000 x g, 4°C for 10 min. acidify the supernatant containing biosurfactant with 6N HCl until pH 2.0 is obtained.

Solvent Extraction:

Extract an acidified supernatant with the equal volume of mixture of the extraction solvents with the following ratio; (chloroform: n-butanol; 65:15). Shake the mixture vigorously for 15 min and allowed to set until the phase separation occurs. White coloured precipitate if seen at the interface between the two liquids proved the presence of biosurfactant. The biosurfactant formed was carefully taken out with the help of micropipette and kept in centrifuge tubes. These were centrifuged at 7000rpm, 4°C for 30 minutes. The supernatant was discarded and the pellet obtained was the crude extract of biosurfactant.

Determination of in vitro antifungal activity of extracted biosurfactant

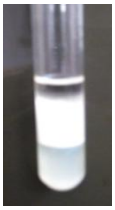
Sterile Muller Hinton Agar media was prepared and poured into sterile petriplate. Plate was swabbed with *Candida albicans* culture. Three Wells were made using alcohol sterilized cork borer and were named as positive control (1%SDS), Negative control (Sterile Distilled water), Test (Extracted Biosurfactant). Plates were kept in incubation at 28°C for 24-48 hours.

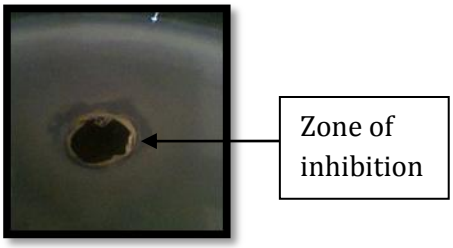
RESULTS AND DISCUSSION

Production and extraction of biosurfactant

After 5 days of incubation medium was dispensed into centrifuge tubes and was centrifuged at 10000g at 4^{0}c for 10 min and the resultant supernatant was extracted using chloroform and n-butanol solvent mixture in the ratio 65:15.White colored precipitation was seen at the interface between the two liquids proved the presence of biosurfactant. The biosurfactant formed was carefully taken out with the help of micropipette.

Table 1: Confirmation of biosurfactant production by *Pseudomonas aeruginosa*

TEST	OBSERVATION	RESULT
Phenol-Sulphuric Acid Method	Orange colour developed indicated the presence of glycolipids.	Positive 
Blue agar plate method (BAP	A dark blue zone around the well indicated the presence anionic biosurfactant.	Positive 
Emulsification test (E24)	63.75.% emulsion was observed	Positive 



Determination of in vitro antifungal activity of extracted biosurfactant

The crude extract of biosurfactant showed antifungal activity against *Candida albicans* after 48hrs. The presence of clear zone marked the antifungal activity of biosurfactant.

CONCLUSION

In conclusion, the study represented surfactant activity of the bacterial strain used. This confirms *Pseudomonas aeruginosa* shows biosurfactant producing ability by utilizing Oilve oil as substrate. Used simple extraction method is efficient in extracting biosurfactant. Extracted biosrfactant is showing antifungal activity in vitro hence study suggests its use as a therapeutic agent. Further study recommends in vivo antifungal activity on animal and human cell lines along with further purification of biosurfactant.

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