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Research Article

INVESTIGATION ON ANTIMICROBIAL ACTIVITY OF BIOSURFACTANT PRODUCED BY *PSEUDOMONAS FLUORESCENS* ISOLATED FROM MANGROVE ECOSYSTEM

Govindammal M*[1] and Parthasarathi R[2]
[1]Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, India
[2]Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, India

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*Email: govimscbiotech1@gmail.com

ABSTRACT
The aim of this present study is to investigate the antimicrobial activity of rhamnolipid biosurfactant produced by *Pseudomonas fluorescens* MFS03 isolated from mangrove forest soil using groundnut oil cake as substrate. The biosurfactant was extracted with an equal amount of ethyl acetate and the concentrated extract was subjected to FT-IR analysis. The important adsorption bands at 3466.24, 2926.45, 1743.47, 1407.30 and 1162.26 cm^{-1}indicate the chemical structure of rhamnolipid. The rhamnolipid biosurfactant was investigated for the potential antimicrobial activity by using disc-diffusion method against Gram positive bacteria (*Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, Methicillin resistance *S. aureus*) Gram negative bacteria (*Escherichia coli*, *Salmonella typhimurium*) and a yeast (*Candida albicans*). The biosurfactant showed distinct antibacterial activity towards tested bacteria and shows an antifungal activity against yeast. The biosurfactant with different concentration was performed for the evaluation of antimicrobial activity. Maximum antimicrobial activity of the biosurfactant (50µl) was observed in *S. aureus* (23 mm) and it was found that the biosurfactant activity was dependent on the concentration. So it could be used as a therapeutic agent in pharmaceutical application.
KEY WORDS: Biosurfactants, antimicrobial activity, Rhamnolipid, Mangrove forest soil, groundnut cake.

INTRODUCTION

Biosurfactants are amphiphilic molecules that have both hydrophilic and hydrophobic moieties which partition preferentially at the interfaces such as liquid/liquid, gas/liquid or solid/liquid interfaces. Such characteristics enable emulsifying, foaming, detergency and dispersing properties. They are categorized by their chemical composition and microbial origin, which includes glycolipid, lipopeptides, polysaccharides-protein complexes, protein-like substances, lipopolysaccharides, phospholipids, fatty acids and neutral lipids[1].Their low toxicity and environmental friendly nature and the wide range of potential industrial applications in bioremediation, health care, oil and food processing industries makes them a highly sought after group of chemical compounds. Interest in them has also been encouraged because of the potential advantages they offer over their synthetic counterparts in many fields spanning environmental, food, biomedical, petrochemical and other industrial applications. Therefore, it is reasonable to expect diverse properties and physiological functions of biosurfactants such as increasing the surface areas and bioavilability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, quorum sensing, and biofilm formation[2].

The antimicrobial activity of several biosurfactants has been reported in the literature for many different applications[3]. Further-more Rhamnolipid has demonstrated antimicrobial activity against several microorganisms such as *Clostridium perfringens*, *Salmonella typhimurium, Escherichia coli, Enterobacter aerogenes, Phytophthora infestans, Phytophthora capsici, Botrytis cinerea, Fusarium graminearum* and *Mucor* spp.[4,5,6] .

The aim of the present study was to investigate the antimicrobial effect of the biosurfactant produced by *Pseudomonas fluorescens* MFS03 isolated from mangrove forest soil using groundnut oil cake as substrate.

MATERIALS AND METHODS

Microorganism:

Pseudomonas fluorescence MFS03 was isolated from Mangrove forest soil, Pitchavaram, India using Mineral salt medium supplemented with 0.1% (v/v) of crude oil and the species level is identified by following Bergey's Manual of Determinative Bacteriology[7].

Determination of biosurfactant producing ability:

Biosurfactant production was examined with drop collapsing test[8],Oil spreading test [9], CTAB plate assay[10], Haemolytic activity[11] was detected as the occurrence of a define clear zone around the colony. Lipase activity was measured using the tributyrin agar plates as described by[12]. Emulsification activity was performed according to[13]. The entire assays were performed in triplicates.

Production Media and growth conditions

The potential biosurfactant producer was cultured in fermentation medium which contains (g/L^{-1}): 1.0 K_2HPO_4, 0.2 $MgSO_4.7H_2O$, 0.05 $FeSO_47H_2O$, 0.1 $CaCl_2.2H_2O$, 0.01 $Na_2MoO_4.2H_2O$, 30 NaCl and 5 g of dried groundnut oil cake were added and autoclaved at 120 ^{0} C for 15 min. The pH was maintained at 7.0, the sterilized medium was inoculated with 5 ml (x 10^9 cells per ml) of culture broth and the content were mixed properly and incubated at 35 ^{0} C for 3 days in an orbital rotary shaker set at 120 rpm min^{-1}.

Extraction of biosurfactant:

The culture broth was centrifuged at 12,000 rpm for 15 min to remove the cells as well as debris and the supernatant was filtered through 0.2 µm filter. Filtered supernatant was used for the extraction of biosurfactants. Extraction was performed by acid precipitation followed by liquid-liquid extraction. The cell free supernatant was acidified with concentrated HCl to attain a pH 2.0 and extracted with an equal volume of solvent such as ethyl acetate, diethyl ether and acetone. The resultant aliquot was concentrated to dryness in a rotary vacuum evaporator (Rotavapor R-205; Buchi, Bern, Switzerland) and tested for the emulsification activity.

FTIR spectral analysis of biosurfactant

The FT-IR spectra was recorded in a Thermo Niocolet, AVATAR 330 FT-IR system, Madison WI 53711-4495, in the spectral region of 4000-400 cm^{-1} using potassium bromide (KBr) solid cells. The analysis was done in the Department of Chemistry, Annamalai University, India. The concentrated extract from *Pseudomonas fluorescens* MFS03 was subjected to FT-IR. The spectra were recorded and analyzed using the standard methods described by the previous authors[14,15].

Antimicrobial assay:

The antimicrobial activity of extracted compound was studied against different gram positive and gram negative bacteria and yeast (Table 1). The sterile whatmann No1 filter paper (0.6 cm) soaked with biosurfactant solution in methanol (10, 20, 30, 40, 50 µg/ml) was assayed on the surface of an nutrient agar and malt extract media for bacteria and yeast, respectively inoculated with the tested organisms. After the incubation period for 24 h at 37 ± 2 ^{0} C and for 48 h 25 ± 2 ^{0} C for bacteria and yeast, respectively, the diameter of inhibition zones was measured[16]. Negative controls were prepared using the same solvents as employed to obtain the extract. Ofloxacin (5 µg, sigma) for gram-positive bacteria, Cefaperazone-sulbactam (5 µg, Sigma) for gram-negative bacteria and amphotericin B (5 µg, Sigma) for yeast were used as positive controls. To ensure that, the results were reproducible, the average of three independent measurements was taken.

Table 1: Antimicrobial activity of the biosurfactant produced by *Pseudomonas fluorescens* MFS03

Tested organisms	Zone of inhibition diameter (mm) with different concentration of biosurfactant (µl)				
	10	20	30	40	50
Gram Positive bacteria:					
Bacillus subtilis	15 ± 0.56	16 ± 0.89	18 ± 0.25	20 ± 0.16	21 ± 0.14
Listeria monocytogenes	14 ± 0.62	15 ± 0.36	16 ± 0.17	17 ± 0.12	18 ± 0.12
Staphylococcus aureus	17 ± 0.14	19 ± 0.68	20 ± 0.23	21 ± 0.75	23 ± 0.26
Methicillin-resistant *S.aureus* (MRSA)	14 ± 0.16	15 ± 0.11	16 ± o.45	17 ±o.67	18± 0.23
Gram Negative bacteria:					
Eschercia coli	10 ± 0.11	11 ± 0.20	12 ± 0.02	14 ± 0.23	15 ± 0.35
Salmonella typhimurium	7 ± 0.12	9 ± 0.86	10 ± 0.08	11± 0.96	12 ± 0.26
Candida albicans	9 ± 0.23	10 ± 0.75	11± 0.56	13 ± 0.25	14 ± 0.23

Values are means ± S.D (mm) of three separate experiments.

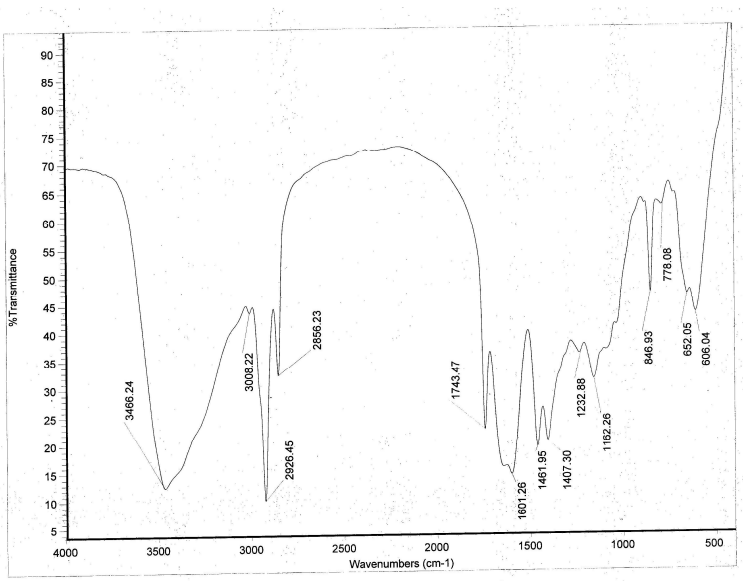

Figure 1: FT-IR spectral analysis of the biosurfactant produced by *Pseudomonas fluorescens MFS03.*

RESULT AND DISCUSSION

Characterization of Biosurfactant:

In the present study, the possibility of biosurfactant production by *Pseudomonas fluorescens* MFS03 isolated from Mangrove forest soil, Pitchavaram, Tamil Nadu, India using cheaper renewable substrate, groundnut oil cake and its antimicrobial activity of biosurfactant against clinical pathogens were reported. The production of biosurfactant using cheaper carbon sources was already reported by earlier studies, plant-derived oils, oil wastes, starchy substances, molasses, cashew apple juice and agriculture residues[17-22] which has been supporting the present study on use of renewable substrates for the biosurfactant production.

The isolate *Pseudomonas fluorescens* MFS03 shows positive results for all the five screening method tested. Depending on the screening test performed *Pseudomonas fluorescens* MFS03 was confirmed as biosurfactant producing strain and selected for the further studies. FT-IR analysis of the biosurfactant showed that, the most important adsorption bands located at 3466.24 (OH bond, typical polysaccharides), 2926.45 and 2856.23(CH band: CH_2-CH_3, hydrocarbon chains), 1743.47 and1601.26 cm^{-1}(for C=O, C=O ester bond), 1407.30 cm^{-1}(C-N amide groups). The C-O stretching bands at 1162.26-1232.88 cm^{-1} confirm the presence of bonds formed between carbon atoms and hydroxyl groups in the chemical structures of the rhamnose rings and 846.93,652.05(for the CH_2 groups) (Fig1). Therefore, it can be concluded that the biosurfactant produced by *Pseudomonas fluorescence* MFS03 *is* a rhamnolipid structure. (Fig. 1).The allocation conspicuous absorption in the spectra obtained corresponded to the characteristic group absorption of rhamnolipids[23,24,25].

Antimicrobial activity of biosurfactant:

One useful property of many biosurfactant that has not been reviewed extensively is their antimicrobial activity. Other medical relevant uses of biosurfactants include their role as anti-adhesive agents to pathogens, making them useful for treating many diseases and as therapeutic agents[2] . The rhamnolipid biosurfactant produced by *Pseudomonas fluorescens* MFS03 exhibited interesting antimicrobial activities and the results were listed in Table 1. The antimicrobial activity of rhamnolipids has been described for many bacteria[4,5]. The antimicrobial effect of biosurfactants is the adhering property of biosurfactants to cell surfaces caused deterioration in the integrity of cell membrane and also breakdown in the nutrition cycle[26].

CONCLUSION

Our findings have demonstrated on the production of biosurfactant using renewable substrate and the potential antimicrobial activity of rhamnolipid biosurfactant against various clinical pathogens. It also showed relatively high antibacterial activity against methicillin resistant *S. aureus* (MRSA). So, it can be used in pharmaceuticals or in cosmetics for dermal applications.

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