Low Cost Production Of Biosurfactant From Different Substrates And Their Comparative Study With Commercially Available Chemical Surfactant.

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Abstract: Biosurfactants are amphiphilic compounds produced by various bacteria and fungi which reduce surface and interfacial tension. In this work, the biosurfactant produced by *Bacillus subtilis* strain isolated from soil samples was characterized and its properties compared with commercially available chemical surfactants. *Bacillus subtilis*was used for the production of biosurfactant and its activity was tested against crude vegetable oil. The crude biosurfactant was produced using four different substrates and its emulsification activity was compared against sodium dodecyl sulphate (SDS). The results showed that the isolated bio surfactant from coconut waste showed the highest emulsification activity even more than sodium dodecyl sulphate (SDS) which is a commercial chemical surfactant. Furthermore its antimicrobial activity was checked against *Staphylococcus aureus, Escherichia coli, Pseudomonas and Salmonella typhimurium.* The study concludes that coconut and soyabean waste are the most ideal substrate for biosurfactant biosynthesis, which may have potential industrial applications.

Keywords*:* Biosurfactant, *Bacillus subtilis,* Emulsification, Waste Management

1. INTRODUCTION

In recent years industries have generated a large amount of tropical agricultural residues. Their disposal causes several environmental problem therefore there has been an increasing trend towards more efficient utilization of agro industrial residues like oil cakes, wheat bran, soya bean waste, sesame waste, coconut waste, bagasses etc. These residual by-products serve as an ideal substrate for fermentation processes to produce different commercially important compounds.[1] Mostly agricultural products are utilized as source of raw material as they are produced in large quantities, contain large amount of usable proteins and carbohydrates with some amount of oil residues, no storage problem, easily available and cheap. Surfactants are molecules that concentrate at interfaces and decrease surface and interfacial tension [2]. These compounds find applications in an extremely wide variety of industrial processes involving emulsification, foaming, detergency, wetting, dispersing or solubilization [3, 4]. However, naturally occurring surface-active compounds derived from microorganisms, also called biosurfactants, are attracting attention as they offer several advantages over chemical surfactants, such as low toxicity, inherent good biodegradability and ecological acceptability [5].

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Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membranes by a variety of yeast, bacteria and filamentous fungi [6, 7] from various substances including sugars, oils and wastes. The unique properties of biosurfactants allow their use and possible replacement of chemically synthesized surfactants in a number of industrial operations [8]. Biosurfactants reduce surface tension, Critical Micelle Concentration (CMC) and interfacial tension in both aqueous solutions and hydrocarbon mixtures [9, 10] In the present study, we have optimized the production of biosurfactant from *Bacillus subtilis* and characterized it. Investigation was carried out on its emulsification capacity as bio preservative in food. Different parameters were tested to optimize for the growth of the strain.

2. MATERIALS AND METHODS

2.1 Microorganism and culturing conditions

The bacterial strain *Bacillus subtilis* was isolated from the garden soil using serial dilution method. Isolated colonies were identified on the basis of morphological and biochemical characteristics. The bacterial strains were enriched in Luria Bertani broth incubated at 37^{o} C for overnight. The culture was streaked on LB slants and stored at 4^{o}C for further use.

2.2 Screening of biosurfactant producing *Bacillus subtilis*

Oil spreading technique (Morikawa*et al.,* 1993) was used to determine the biosurfactant activity of isolated bacteria. 10 µl Crude Oil was added on the surface of 20ml distilled water in Petri dish and diameter of clearing zone indicates surfactant activity [12].

2.3 Enrichment of *Bacillus subtilis*

For enrichment, isolated culturewas transferred to minimal salt medium containing 15 % oil (coconut, sunflower. Castor oil) and incubated for 5 days on rotor shaker at 120 rpm.

2.4 Selection of substrate
The most widely used substrate for biosurfactant production is material of mainly agricultural origin. Therefore in present work we used Basal medium, Coconut waste, Soyabean waste & sesame waste.

2.5 Production and extraction of biosurfactant
The four different substrates such as Basal medium, Coconut waste medium, Sesame waste medium and soyabean waste were used for biosurfactant production. The substrates were mixed with distilled water in 1:10 ratio and then sterilized. It was then inoculated with overnight grown 3% inoculum and incubated for 48hrs at 37° C. After incubation period culture media was centrifuged at 10,000rpm for 20 minutes and supernatant was used as crude source. In order to precipitate lipid and proteins HCl was added to the supernatant up to pH 2.0 and kept overnight at 4°C [13]. It was again centrifuged at 10,000 rpm for 20mins. Grey white precipitate was collected. For further extraction 10mL of Chloroform and methanol (2:1 v/v) was added to the precipitate and incubated in rotatory shaker at 30^{0}C for 15mins at 250 rpm agitation. The content was centrifuged at 10,000rpm for 20mins and supernatant was evaporated by air drying. The remaining residue was dispensed in Sodium Phosphate buffer (pH 7.0) and stored at 4^{0}C [11].

2.6 Chemical characterization
Preliminary characterization of the biosurfactant was done by Thin layer Chromatography (TLC). The components were separated on Silica gel 60 plate (Merck) using Chloroform: Methanol: water (65:15:1). Ninhydrin was used as spraying agent and plate was heated at110^0 for 5mins. (Makkar and Cameotra) [14]. Rf value of Surfactin (sigma) was used as standard.

2.7 Biochemical characterization

2.7.1 Determination of emulsification activity
The emulsification potential was carried out using modified method of Cameotra et al. 2004 [15]. The extracted surfactant (0.5 mL) was added to a screw capped tube containing 7.5ml of Tris-Mg buffer (20mM TrisHCl (pH 7.0) and 10 mM MgSO4) and 0.1 mL of edible vegetable oil. After a vigorous vortex, the tubes were allowed to stand for one hour. Absorbance was measured at 540nm.

2.7.2 Antimicrobial activity
The antimicrobial activity of the produced biosurfactant from four different substrates was studied against different common human pathogens. The antimicrobial activity was evaluated by agar well diffusion method. After incubation period for 48 h at 37°c zones of inhibition are measured.

2.7.3 Protein and carbohydrate estimation
Protein estimation was carried out by Lowry et al. (16) method using bovine serum albumin as standard. For carbohydrate estimation DNS method is used based on the principle that reducing sugar has the property to reduce many of the reagents.

2.7.4 Comparison of biosurfactant with commercially available surfactant (SDS)
The emulsification activities of produced biosurfactant using four different substrates were compared with commercially available surfactant such as SDS.

3. RESULTS AND DISCUSSION
According to Bergey's manual of Determinative Bacteriology (Bergey's and Holt, 1994). Morphological and Biochemical test are given which indicated that the most probable identity of the isolate is *Bacillus subtilis*

3.1 Chemical characterization of biosurfactant by TLC
Preliminary characterization of biosurfactant using TLC revealed a blue color spot and the $R_f$value of experimental samples were calculated & compared with standard sigma surfactine.

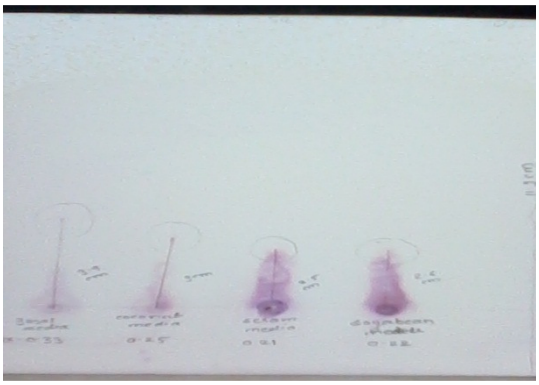


Figure 1. Rf Values of biosurfactants from the four substrates by TLC.

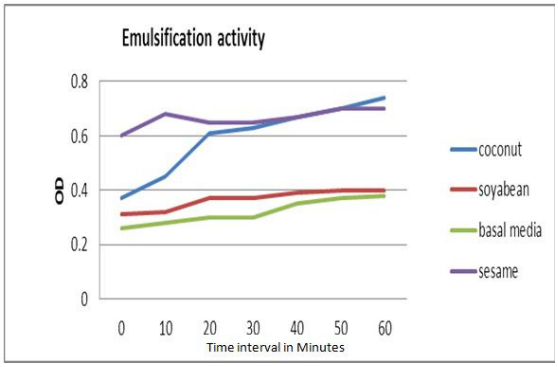
Table 1: The R_f values of biosurfactant and sigma surfactine

Sample	R_f value
Sample A(coconut waste)	0.33
Sample B(soyabean waste)	0.25
Sample C(basal medium)	0.21
Sample D(sesame waste)	0.22
Sigma surfactine	0.5

3.2 Biochemical characterization of Biosurfactant

3.2.1 Determination of Emulsification Activity:
Highest emulsification activity was observed for coconut waste after 60 minutes as seen in graph 1 below. However, the average activity in one hourof sesame waste was the highest as compared to the others. Basal media and soyabean waste showed the lowest activity.

Graph 1: Emulsification activity plotted against Time V/S OD.



3.2.2 Antimicrobial Activity:

The antimicrobial activity of the produced biosurfactant using four different substrates was observed against different pathogens such as *E. coli, P. aeroginosa, S. typhimurium* and *S. aureus.* Biosurfactant using soyabean substrates shows highest antimicrobial activity as compare d to using substrates such as coconut, sesame and basal medium.

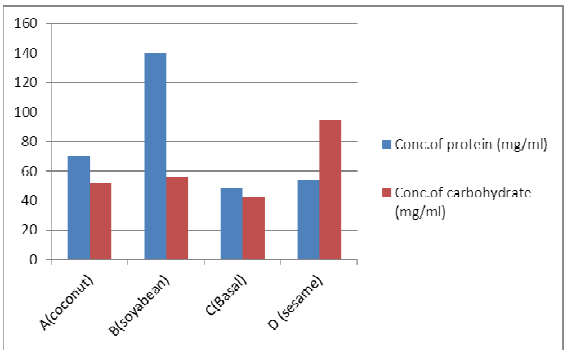
Table 3: Antimicrobial activity

Microorganisms	Coconut	Soyabean	Basal medium	Sesame
E. coli	15mm	28mm	18mm	17mm
Pseudomonas	24mm	28mm	21mm	20mm
S. typhimurium	18mm	30mm	20mm	17mm
S. aurues	16mm	25mm	25mm	15mm

3.2.3 Protein and carbohydrate estimation

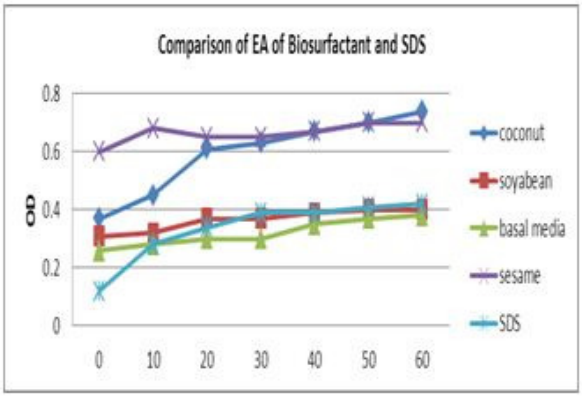
Protein estimation was carried out by using the Lowry method. The highest protein content observed in biosurfactant produced using soyabean as substrates (140 mg/ml). For carbohydrate estimation DNS method is used. The biosurfactant produced using sesame shows highest carbohydrate content (94 mg/ml).

Graph 2: Protein and carbohydrate content of produced biosurfactant using four different substrates.



3.2.4 Comparison of biosurfactant and SDS by determining Emulsification activity:

The comparative chart of emulsification activity of produced biosurfactant using four different with commercially available chemical surfactant such as SDS. The maximum emulsification activity was observed for biosurfactant produced using coconut as substrates as compared to commercially available chemical surfactant such as SDS.



This study revealed the possibility of biosurfactant production using cheaper carbon and protein sources like waste soyabean, coconut waste, sesame waste, biosurfactant produced in the present study using different agriculture waste containing trace amount of oil showed good emulsification activity against four different oils. Further, it encouraged the aim of the present study to produce biosurfactants from cheaper carbon sources with high emulsification property. The emulsification activity of biosurfactant used in this study was higher than the emulsification activity recorded with SDS. while considering the advantages of biosurfactant over chemically synthesized surfactants, such as lower toxicity, biodegradability and ecological acceptability the possibility of replacing the chemical surfactant in oil pollution with biosurfactant is sought and need further research with different kind of experiments.

4. CONCLUSION

The present study is an attempt to find economically cheaper carbon sources for the large scale production of microbial biosurfactant. Results obtained in biosurfactant production with agriculture waste suggested the possibility of industrial production of biosurfactant using economically cheaper carbon sources. Satisfactory emulsification activity of the biosurfactant against eight different oils indicated its diverse applicability against different oil pollution. Further purification, structural characterization of biosurfactant and genetic regulation of biosurfactant production are in progress.

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