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Original Research Paper

Microbiology

PRODUCTION AND CHARACTERIZATION OF BIOSURFACTANTS FROM BACILLUS SUBTILIS

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ABSTRACT Surfactants are the substances that lowers the surface tension or interfacial tension between liquid & liquid (or) liquid & solid . These substances tend to form micelles in a solution (i.e either in water or oil phase) and absorb to interface between gases / solids. There are two groups of surfactant molecules hydrophilic group that has affinity towards to water and hydrophobic group which does not show affinity to water. Based upon the polarity composition of head group surfactants are classified into cationic, anionic, non-ionic and amphoteric (zwitterionic) Ionic surfactants are divided into cationic surfactants and anionic surfactants whereas non-ionic surfactants are subclassified according to the type of hydrophilic groups. Mainly surfactants act as a wetting agents, foaming agents and emulsifiers. Surfactants also play important role in practical applications and products that includes shampoos, tooth pastes, inks, foams and nonoxynol-9

KEYWORDS : surfactants, wetting agents, foaming agents, emulsifiers

INTRODUCTION

Microbial Surfactant (or) Biosurfactants are the compounds that possess both hydrophilic and lipophilic properties. They are said to be amphiphilic compounds in nature. The common amphiphilic molecules are soaps, detergents and surfactants). The special structure of this molecules leads to high solubility in both polar and non-polar solvents. This amphiphilic molecule forms an aggregate of surfactant molecules when they are dispersed in a liquid forming a mixture. Mostly surfactants are produced by microbes, majority is produced by bacteria. Charge of biosurfactant is either neutral (or) negatively charged. (Cameotra & R.S.Makkar 1998). Biosurfactants (or) Microbial Surfactants are classified according to their Molecular Weight (MW) and Mode of Action. Here, media composition plays an important role in the type and quantity of biosurfactant that is produced. (Singh *et al.,* 2018). Comparing with Chemical Surfactants, Microbial Surfactants has several advantages like environmental compatibility, production, specific activity for growing microbes at high temperature and also, they produce less toxicity (Ligia R. Rodrigues 2015). The main factors that affect the biosurfactant production are carbon sources, nitrogen sources, salt concentration, aeration and agitation (Fakruddin Md 2012). The most promising strategy for the production of biosurfactant is solid state fermentation mainly for the foam production that has been mainly indulged in submerged fermentation (P.Singh *et al.*, 2018).

Application of Biosurfactants has been increased world-wide in areas of agriculture, commercial laundry detergents, biopesticide, anti-microbial activity, anti-cancer activity, anti-adhesive agents, antiviral activity and immunological activities. In agriculture, surfactants are used to obtain even distribution of fertilizers in the soil and also promote evenly spreading. Almost all the surfactants have the good stability with commercial laundry detergents that favors the formulation. It has been found that biosurfactants have the ability to inhibit the adherence pestilent organisms to the infection site. Biosurfactants usually plays a very good role in food formulation where the shelf lives of starch containing products are improved. Manufacturing petroleum extraction and petrochemical by biosurfactants are the main application in microbial enhanced oil recovery. These microbial surfactants are easily degraded where they are considered to be less toxic (Fakruddin , 2012). In the present study *Bacillus megaterium* was used for screening and partially purified to characterize for the physical and chemical methods. Nano-vesicle synthesis is carried out and checked for the entrapment efficiency of the synthesized drug and evaluation of the synthesized drug.

MATERIALS AND METHODS

Inoculum Preparation

Bacillus megaterium ATCC 14581 was used in this study. A loopful of *Bacillus megaterium* stock culture was inoculated into a 25ml nutrient rich medium LB (Luria-Bertani). The flask was incubated for 24 hours at room temperature in rotatory shaker (250rpm). Minimal Salt broth was prepared to which 5ml of mixed oil was added the medium was adjusted pH 7 using 100% ethanol. Then the medium was sterilized at 121°C for 15 minutes at 15lbs pressure. After sterilization the broth was inoculated with *B.megaterium*. Inoculated medium was then incubated in a rotary shaker at 250 rpm for 5 days at 30°C. After fermentation bacterial cells were removed by centrifugation at 6000 rpm for 20 min. Supernatant was collected and stored for further process.

Media Production

Minimal Salt broth was prepared to which 5ml of mixed oil was added the medium was adjusted pH 7 using 100% ethanol. Then the medium was sterilized at 121°C for 15 minutes at 15lbs pressure. After sterilization the broth was inoculated with *B.megaterium*. Inoculated medium was then incubated in a rotary shaker at 250 rpm for 5 days at 30°C .

Isolation of Biosurfactant

After fermentation bacterial cells were removed by centrifugation at 6000 rpm for 20 min. Supernatant was collected and stored for further process.

Screening of Surfactant

Oil Displacement test

Screening of biosurfactant production was carried out by Oil Displacement test. In this method 10ml of distilled water was added on to the petri dish and 1ml of mixed oil was added in such a way that oil spreads uniformly on the water surface. Then, 500μL culture supernatant was gently added onto the petri dish.

Partial Purification:

Acid Precipitation

Cell free supernatant was acidified by adding 6N HCL (4.3752 grams) in 20mL of supernatant and pH was adjusted to 2.0. Acidified cell broth was kept overnight at 4°**C** for the precipitation of biosurfactant. The precipitated biosurfactant was collected by centrifugation (6000 rpm for 10 min) and pH

was checked and adjusted to 8.0 and the biosurfactant solution was lyophilized with distilled water. This extract was used for further analysis and purification process. Characterization of Biosurfactant was done by HPLC & FTIR analysis.

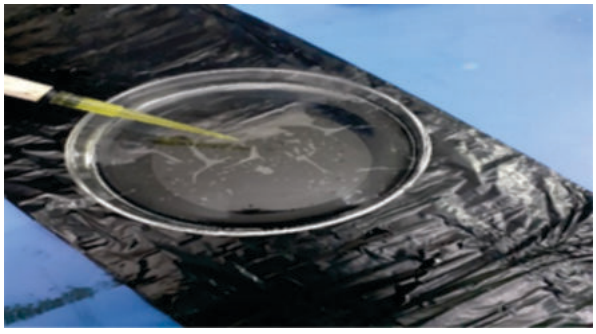
Emulsification Index

EI percentage was estimated by adding 2mL of mixed oil with 2mL of supernatant (separately) into test tube and vortexed. The prepared mixture was vortexed for about 15-20 min at room temperature and left unscathed for 24 hrs and the EI value was collected by measuring the Emulsification Index percentage (H. Dhivya *et al*., 2014).

RESULT AND DISCUSSION

Oil Displacement Test

A thin membrane of oil was formed immediately upon which clear halo zone is formed in the addition of surfactant. Oil Displacement Test is the rapid method for the selection of biosurfactant producers and the results revealed the positive analysis. This shows that *Bacillus megaterium* has very good surfactant activity (Obula Reddy Chittepu 2019).



Emulsification index

Emulsification Index was performed to check the presence of surfactant activity. The emulsification activity is defined as the height of emulsion layer divided by the total height and expressed as percentage. Emulsification activity was measured according to the method of Cooper and Golden berg (1987). Mixed oil was used as a carbon source for surfactant production and *Bacillus megaterium* showed emulsification index of 64.86%.

Characterization of Biosurfactant

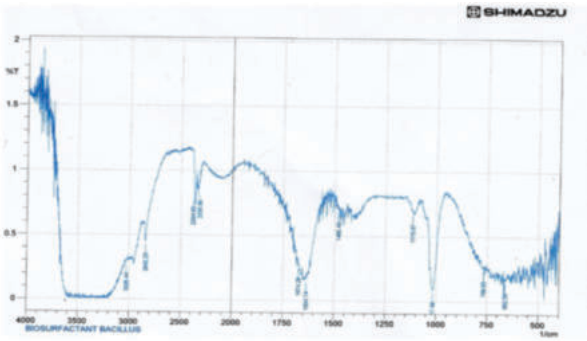
HPLC Analysis

Methanol extraction of the surfactant was used for the partial purification (acid preparation) to characterize the surfactant and to disrupt the micelle structure. The methanol extracted mixture of lipoproteins was resolved with the help of HPLC. The surfactant after partial purification was mixed with methanol and was analyzed. Thus, the retention time was observed to be 1.594 minutes (C.Sivapathasekaran *et al.*, 2009).

FTIR Analysis

Surface active compounds from Bacillus Megaterium was absorbed from between 500-4000 wavenumber cm-1 and transmittance are 662.58 cm-1,2842.83 cm-1,364.83 cm-1,1674.28 cm-1,1634.74 cm-1,1480.43 cm-1,1115.87 cm-1,1017.49 cm-1,769.63 cm-1,662.58 cm-1. All Transmittance peak data's were measured using standard IR values. The peak 3026.44 relates to the aromatic group. The peak 2842.83 belongs to carboxylic acid.

The peak 1674.28 belong to general form of carbonyl functional group. Primary amine corresponds to the peak 1634.74 and the peak 1480.43 corresponds to nitro compounds group. In all the fractions the peaks observed from 1115.85 – 662.58 actively belong to the alkyl and aryl-halides group (Obula Reddy Chittepu 2019).



CONCLUSIONS

Despite an enormous amount of research work in the last two decades the production of biosurfactants and their commercial success compared to their synthetic counterparts still remains an economic challenge. Microbial surfactants are not yet competitive with chemical ones, but efforts should be made on different aspects. *Bacillus megaterium* is rich in potential and usefulness in the years to come. An efficient HPLC & FTIR method was developed and optimized for the analysis and purification of lipoprotein biosurfactant produced by *Bacillus megaterium*.

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