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PRODUCTION OF BIOSURFACTANT FROM *BACILLUS SUBTILIS* MTCC 441

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ABSTRACT

Biosurfactants are amphiphilic biological compounds produced extracellularly as a part of cell membranes by a variety of yeast, bacteria and filamentous fungi. Specifically, lipopeptide biosurfactants are produced by Bacillus subtilis species and are classified into three types: surfactin, iturin and fengycins. The biochemical mechanism for their biosynthesis depends upon non-ribosomal peptide synthetases. Particularly, surfactin synthesis is dependent upon surfactin synthetase operon which is regulated by complex cascade of reactions. In this study, the aim is to produce surfactin from Bacillus subtilis MTCC 441 using the production medium. The surfactin was produced by inoculating the culture in the production medium for 5 days and subsequent centrifugation.The presence of surfactin was determined qualitatively using oil drop spread assay. Further, synergistic activity of biosurfactant along with antibiotics & silver nanoparticles is tested against Staphylococcus aureus and Candida albicans.

KEY WORDS

Biosurfactants, lipopeptides, surfactin, production medium, oil drop spread assay,

INTRODUCTION:

Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membranes by a variety of yeast, bacteria and filamentous fungi 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. They reduce surface tension and interfacial tension in both aqueous solutions and hydrocarbon mixtures. Biosurfactants display a wide variety of chemical structures, including glycolipids, lipopeptides, phospholipids, fatty acids, or neutral lipids, among others. Biosurfactants have a special advantage over the chemical surfactants, such as lower toxicity, higher biodegradability, biocompatibility and digestibility, better environmental compatibility, higher foaming, high selectivity, effectiveness at extremes of pH, temperature, salinity and widespread applicability, and their unique structures which provide new properties that chemical surfactants may lack. [(1)]

Biosurfactants has many applications in the areas such as food and food related industries (as

emulsifiers, foaming, wetting, solubilizers, anti-adhesive agents), biomedicine and therapeutics. Some biosurfactants are a suitable alternative to synthesized medicines and may be used as safe and effective therapeutic agents (Singh and Cameotra, 2004). The antimicrobial activity of several biosurfactants has been reported against bacteria, fungi, algae and viruses. [(1)]

Lipopeptides form the most widely reported class of biosurfactants having antimicrobial action. Among the lipopeptides, surfactin, produced by *Bacillus subtilis* is the first and the most well-known member. Surfactin is a bacterial cyclic lipopeptide, largely prominent for its exceptional surfactant power. Its amphiphilic properties help this substance to survive in both hydrophilic and hydrophobic environments. It is an antibiotic produced by the Gram-positive endospore-forming bacteria *Bacillus subtilis*. In the course of various studies of its properties, surfactin was found to exhibit effective characteristics like antibacterial, antiviral, antifungal properties.

In this study, the aim is to produce biosurfactant surfactin from *Bacillus subtilis MTCC 441* using production medium broth as the production medium.

LITERATURE SURVEY:

1. **Mohammad Irfan, Sushil Kumar Shahi, P.K. Sharma (2015)** ***In vitro synergistic effect of biosurfactant produced by Bacillus subtilis MTCC 441 against drug resistant Staphylococcus aureus.***

Microorganisms are capable to produce a wide range of surface active metabolites which are known as biosurfactant. In the present study, the production of biosurfactant from Bacillus subtilis MTCC 441 and evaluation of its synergistic activities with known antibiotics against drug resistance Staphylococcus aureus were performed. Drops collapse grid method was developed for detection of biosurfactant production in the medium. The production of biosurfactant was done in previously defined production medium. The extraction of biosurfactant was done in chloroform: methanol (2:1 v/v) solvent. The synergistic activity of crude biosurfactant was determined by disc diffusion and minimum inhibitory concentration assay. The Staphylococcus aureus was found resistant against ampicillin-sublactum (10µg), ampicillin (25µg), and cloxacillin (5µg), which showed no zone of inhibition but in the combination of crude biosurfactant, the zone of inhibition were measured as 14 mm, 14 mm and 19 mm, respectively. The crude biosurfactant showed good synergy with most of the antibiotics used in the study. The results demonstrated that biosurfactant possesses considerable potentiality to break the resistance of the pathogen tested. In future, it can be used for the development of effective drug against resistant bacteria.

2. **EduardoJ.Gudi˜na et al. (2011)** ***Biosurfactant producing Lactobacilli: Screening, Production profiles and effect of medium composition.***

Biosurfactant production was screened in four lactobacilli strains. The highest biosurfactant production (excreted and cellbound biosurfactants) was achieved with Lactobacillus paracasei ssp. paracasei A20, a strain isolated from a Portuguese dairy plant, with a decrease in the surface tension of 6.4mNm−1 and 22.0mNm−1, respectively. Biosurfactant production by this strain was evaluated under different culture broth compositions. The use of different nitrogen sources revealed that yeast extract is essential for bacterial growth, while peptone is crucial for biosurfactant synthesis. For biosurfactant production, the use of peptone and meat extract yielded a higher production when compared to the standard medium, with a surface tension reduction of 24.5mNm−1. Furthermore, experiments were also conducted in a reactor with pH and temperature control. Biomass and biosurfactant production in bioreactor was higher comparing with the experiments conducted in shake flaks. The optimization procedure adopted in the current work was found to improve cthe biosurfactant production and opened new perspectives for the use of L. paracasei ssp. paracasei A20 as a promising biosurfactant-producer.

Ximenes, E.A. et al. (2007) *Antimicrobial activity of surfactants produced by Bacillus subtilis R14 against multidrug-resistant bacteria.*

A	Quantity (in g)	B	Quantity (in g)
molasses	10.4	Na-EDTA	0.096
$(NH4)_2SO_4$	8.16	$ZnCl_2$	0.16
K_2HPO_4	13.05	$CoCl_2.6H_2O$	0.057
KH_2PO_4	3.84	$Na_2MoO_4.2H_2O$	0.057
$MgSO_4.7H_2O$	0.48	$CaCl_2$	0.403
$NaNO_3$	8.16	$FeSO_4.7H_2O$	2.19

Lipopeptides represent a class of microbial surfactants with increasing scientific, therapeutic and biotechnological interests. The genus Bacillus is a producer of these active compounds, and among them B. subtilis produces surfactin, the most potent biosurfactant known. These compounds can act as antibiotics, antivirals, anti-tumorals, immunomodulators and enzyme inhibitors. In this work, the antimicrobial activity of biosurfactants obtained by cultivation of B. subtilis R14 was investigated against multidrug-resistant bacteria. During cultivation in defined medium, the surface tension of the medium was reduced from 54 mN/m in the beginning of the microbial growth to 30 mN/m after 20 hours. A crude surfactant concentration of 2.0 g/L was obtained after 40 hours of cultivation. A preliminary characterization suggested that two surfactants were produced. The evaluation of the antimicrobial activity of these compounds was carried out against 29 bacteria. Enterococcus faecalis (11 strains), Staphylococcus aureus (6 strains) and Pseudomonas aeruginosa (7 strains) and
Escherichia coli CI 18 (1 strain) displayed a profile of well-defined drug resistance. All strains were sensitive to the surfactants, in particular Enterococcus faecalis. The results demonstrated that lipopeptides have a broad spectrum of action, including antimicrobial activity against microorganisms with multidrug-resistant profiles.

Materials and Methods:

Microorganism:

Bacillus subtilis MTCC 441 was used for the production of biosurfactant surfactin. The culture in its mid log phase must be added in the production medium. Hence, a growth curve was performed to confirm the time this culture takes to reach the mid-log phase. This time was found to be 4 hours.

Production medium: Composition and preparation:

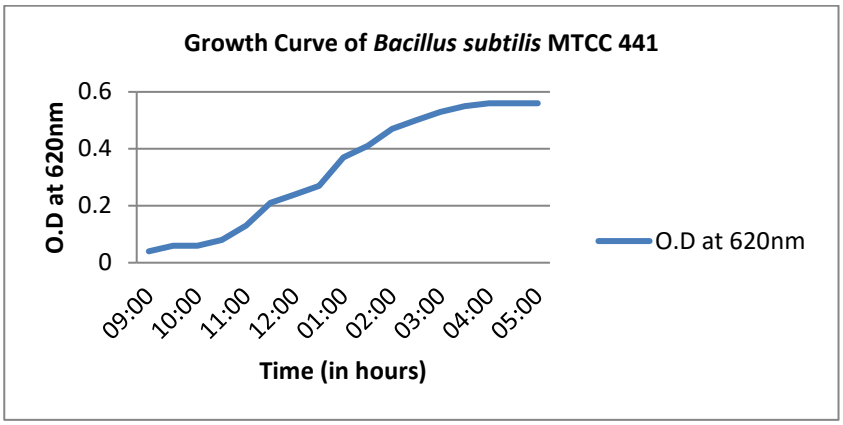
The production medium that is the production medium (1) has the following composition (gram per 250ml):
Both A and B components are prepared in 250ml distilled water in two separate 500ml flasks. Then, 10ml solution from B flask is added to the flask A and is mixed. Following sterilization, the medium is inoculated aseptically with the culture which was brought to mid-log phase. This was further kept for 5 days enrichment at room temperature on a rotary shaker. After 5 days, the broth is made cell-free by centrifugation at 2000rpm for 10mins. Supernatant was then filtered to obtain clear broth.

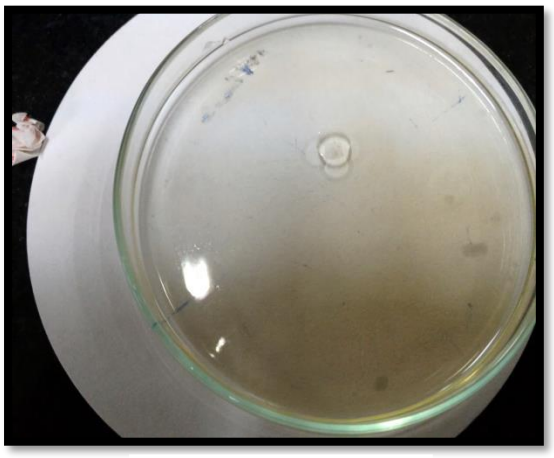
Qualitative Detection of Biosurfactant:

The cell free broth obtained is then tested for presence of biosurfactant by the oil drop spread assay. (1) In a petridish 5ml of water is poured (the petridish is kept on a white background) a drop of cell free broth is added and is allowed to mix. Now, a drop of vegetable oil is added. It was seen that the drop didn't spread (even when kept undisturbed for a very long time) on the surface of water and retained its circular shape. This confirmed that the the presence of biosurfactant in cell free broth. This is because biosurfactant's main function is to alter the surface tension which was seen in this case as the drop retained its shape.

Results:

1) Growth curve result:





Oil drop spread assay

Time (in hours)	O.D at 620nm
9:00	0.04
9:30	0.06
10:00	0.06
10:30	0.08
11:00	0.13
11:30	0.21
12:00	0.24
12:30	0.27
1:00	0.37
1:30	0.41
2:00	0.47
2:30	0.5
3:00	0.53
3:30	0.55
4:00	0.56
4:30	0.56
5:00	0.56

1) Qualitative test (The oil drop spread assay):

The oil drop didn't spread and maintained its shape when added onto the surface of cell free broth and water emulsion. This confirms the presence of biosurfactant surfactin.

CONCLUSION:

Biosurfactant was produced using the previously defined production medium from Bacillus subtilis MTCC 441. The biosurfactant produced was detected qualitatively using oil drop spread assay.

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