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IN VITRO SCREENNING AND CHARACTERIZATION OF BIOSURFACTANT FROM MARINE *STREPTOMYCES* Sp

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ABSTRACT

This study deals with production and characterization of biosurfactant from marine actinomycetes which isolated from east coast region of Nagapattinam beach in Tamil Nadu. The production of the biosurfactant was found to be higher in medium containing sucrose and lower in the medium containing glycerol. Yeast extract was the best nitrogen source for the production of the biosurfactant. The purified biosurfactant was shown strong antimicrobial activity and antioxidant activity. The biosurfactant was produced from the marine *Streptomyces* sp. using non-hydrocarbon substrates such as sucrose that was readily available and not required extensive purification procedure. *Streptomyces* species N11 can be used for microbially enhanced oil recovery process.

KEYWORDS: Biosurfactant, *Streptomyces* sp, antioxidant, Antimicrobial activity.

INTRODUCTION

Biosurfactants are a structurally diverse group of surface active molecules synthesized by microorganisms. Virtually, all the surfactants are chemically synthesized (M. Banat, 1995). Nevertheless, in recent years, much attention has been directed toward biosurfactants owing to their advantages such as low toxicity, high biodegradability, better environmental compatibility, high foaming capability, higher selectivity, specific activity at extreme temperature, pH, salinity, and the ability to synthesize them from renewable food stocks (J.D. Desai, 1997; I. Banat, 1993).

A large variety of biosurfactants have been reported; their type, quantity and quality have been shown to be influenced by many factors such **as** i) the nature of the carbon substrate (Harvey *et al.*, 1990),

Biosurfactants are attracting attention in recent years because they offer several advantages over chemical surfactants due to its low toxicity, inherent good biodegradability and ecological acceptability (Banat, 2000).

Biosurfactant production by actinomycetes has been reported in very few cases. Glycolipid from Rhodococcus erythropolis and Rhodococcus aurantiacus and surface active lipid from Nocardia erythropolis were studied in the literature search (Kokare *et al*., 2007). Among the many classes of biosurfactants, lipopeptides represent a class of microbial surfactants with remarkable surface properties and biological activities, such as surplus crude oil recovery, food-processing, de-emulsification, antimicrobial, antitumor, antiviral, and antiadhesive activities. Surfactin, produced by various *Bacillus subtilis* strains, is one of the most powerful and effective lipopeptide-type biosurfactant.

Biosurfactants are divided into low molecular weight compounds such as glycolipids or lipopeptide and high molecular weight compounds such as polysaccharides, proteins, lipopolysaccharides or lipoproteins. Biosurfactants are found to be secreted in the culture broth or remain adherent to microbial cell surfaces. Several therapeutic and biomedical importance including antibacterial, antifungal, antimycoplasmic, inhibit fibrin clot formation, antitumoral and hemolytic agents are recorded for surface active microorganisms (Cameotra *et al*., 2004). The objective of the present study was production and characterizes the main functional properties of the biosurfactant from marine *Streptomyces*. Characterization included the determination of different factors such as pH and temperature, antioxidant and antimicrobial activity of this biosurfactant was assayed against different microorganisms.

MATERIALS AND METHODS

Isolation and identification of marine actinomycetes strain

Marine sediment samples were collected from the east coast region of Nagapattinam beach, Tamil Nadu, India.

Different marine actinomycetes species were isolated by using selective media such as glycerol yeast extract agar, starch casein agar, and maltose yeast extract agar (Chakraborty, 2009; Kumar, 2007).The isolated strains were screened for biosurfactant production by using different techniques. Identification of biosurfactant producing strain N11 was done by Grams staining, biochemical characterization methods.

Screening methods for potential biosurfactant producers

The potential biosurfactant producer was screened by different method such as hemolytic assay, drop collapsing test, oil displacement test (Kokare, 2007). Maximum biosurfactant producing marine actinomycetes sp. B3 was maintained on glycerol yeast extract agar medium for further study.

Effect of pH, temperature, and sodium chloride on biosurfactant production

Effect of pH and temperature on production of biosurfactant was studied by adjusting the pH and temperature of the basal medium to different levels. Effect of NaCl on biosurfactant production was studied by varying the concentrations of NaCl% (w/v) added to the basal medium. Biosurfactant activity was expressed as percentage relative activity (Ilori, 2005).

Production and purification of biosurfactant

Study on growth of the organism and biosurfactant production in glycerol yeast extract medium with crude oil as sole carbon source was carried out as described by Ilori *et al* (2005) The growth medium contained grams/100 ml: sucrose, 2.0 g; yeast extract, 1 g; KH_2PO_4, 0.53 g; NaCl, 3.0 g; $7H_2O$ and crude oil (2%, v/v) (Mulkin-Philips *et al*., 1947). The pH of the medium was adjusted to 7.0 before sterilization. Trace elements solution (1 ml) of Bauchop and Elsden (Bauchop, 1969) was sterilized separately and added aseptically to the medium. The medium (100 ml) contained in an Erlenmeyer flask (250 ml) was inoculated with the organism and incubated at 28°C with shaking at 150 rpm for 12 days. The culture broth was centrifuged (10,000 rpm, 20 min, 4°C) to remove the cells. The biosurfactant was recovered from the cell free culture supernatant acid precipitation as described by Ilori *et al*. 2005.

Total antioxidant activity

The total antioxidant capacity of biosurfactant from *Streptomycetes* sp was carried out bussing Prieto *et al* (1999) method. One mg/ml of all the sample of various extracts were mixed with reagent solution (sulphuric acid, sodium phosphate and ammonium molybdate solution) followed by incubation and the absorbance was measured at 695nm against a blank using vitamin - C as a standard. The blank contained 1 mL of the reagent solution without the sample. The total antioxidant activity was expressed as the absorbance of the sample. The higher absorbance value indicates the higher antioxidant activity.

Antibacterial activity of the biosurfactant

The antimicrobial activity of the lipopeptide biosurfactant was evaluated on a panel of pathogenic microbial test strains obtain from (Department of Medical Microbiology, Annamalai University, Tamil Nadu, India) including *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Staphylococcus aureus*. The strains were cultured individually on tryptic soy broth at 37°C overnight under aerobic conditionsand the inoculum of each strain was adjusted to a concentration of 108cfu mL¯1(equal to 0.5 McFarland standard). The antimicrobial activity of the biosurfactant was examined against thesepathogenic thesepathogenic strains using Agar well diffusion method and expressed interms of minimum inhibitory concentration (Amsterdam, 1996).

RESULT AND DISCUSSION

Characterization of strain N11

The strain N11 shows good growth in temperature range 25– 45°C in 7 days on glycerol yeast extract agar medium, starch casein agar, and maltose yeast extract agar Chakraborty (2009)**.** The aerial mycelium at maturity formed chains of three to several spores. Spores were non-motile. Initially, colonies were relatively smooth surfaced but later they developed a weft of aerial mycelium that appears to be granular, powdery, or velvety and produce a wide variety of pigments responsible for the color of the vegetative and aerial mycelia. Spores were oval and warty, seen like hairy (Fig.1&2) characterization microscopic study (100x). According to morphological, biochemical characterization (Table-1), the isolated strain was found to be a member of *Streptomyces* genus**.**

Fig.1 Pure culture of N11

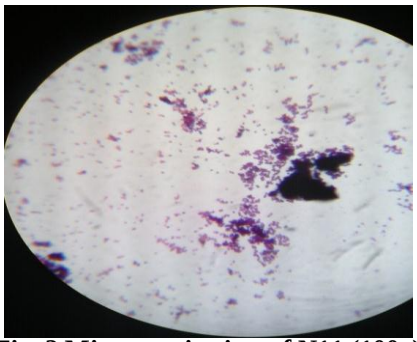

Fig. 2 Microscopic view of N11 (100x)

Table-1 Biochemical Characterization: Isolate-N11

S.NO	TEST	RESULT Isolate N11
1.	Indole test	Positive
2.	Methyl red	Negative
3.	Voges proskauer test	Negative
4.	Citrate utilization	Positive
5.	Urease test	Negative
6.	Catalase test	Positive
7.	Oxidase test	Positive
8.	Starch hydrolysis	Positive

Cultivation conditions and biosurfactant production

The isolated *Streptomyces* sp. N11 produces biosurfactant when grown in various nutrients. The amount of biosurfactant production was varied with respect to media composition. However, the maximum biosurfactant production was observed in glycerol yeast extract. Similar result was observed by Desai and Banat (1997). Therefore, glycerol yeast extract was selected as the biosurfactant production medium for the strain at 28 °C for 9 days.

Screening of biosurfactant production

Hemolytic activity of strain N11 showed zone with diameter 23 mm around the colony. In the present study, a significant correlation was established between the hemolytic activity and biosurfactant production. According to Carrillo *et al*. (1996) and Banat (1993), biosurfactant production of the new isolates was preliminary screened by hemolytic activity. Blood–agar lysis has been used to quantify surfactant and rhamnolipids Chakraborty *et al.* (2009), Carrillo *et al.* (1996) found an association between hemolytic activity and surfactant production, and they recommended the use of blood agar lysis as a primary method to screen biosurfactant production. In drop collapsing test a flat drop and in oil displacement method, a clear was observed (data not shown). From the above observation, it was confirmed that the *Streptomyces* sp. N11 was a potent biosurfactant producer. Both the techniques have several advantages such as small volume of samples was required, rapid and easy to carry out and also do not require specialized equipment Kiran *et al.* (2009).

In total antioxidant activity, the isolate N11 has produced dose dependant manner increasing activity in the range of 78.834±0.001, 80.475±0.001 and 84.323±0.001 respectively (Fig.3)

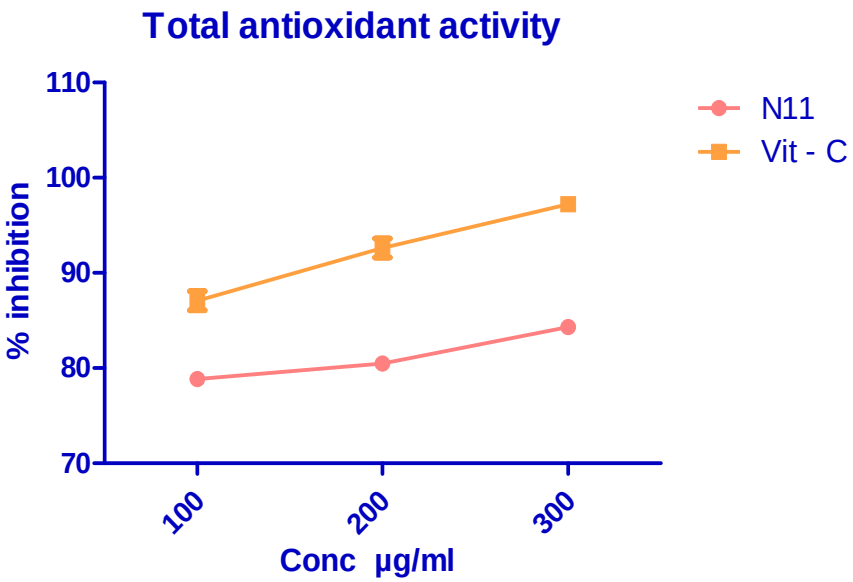

Fig.3 Antioxidant activity of isolate-N11

Antimicrobial activity of biosurfactant

Biosurfactant isolated from *Streptomyces* species showed a wide activity against the pathogenic strains. The partial purified biosurfactant showed activity against *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Staphylococcus aureus* (Table.2). According to Tsuge *et al*. (1996), lipopeptide surfactants are potent antibiotics mainly the surfactin, streptofactin, and gramicidin produced by the microorganism and had the wide antimicrobial activity (Peypoux, 1999), compared to the glycolipid producing strain. A glycolipid surfactant from the *C. antartica* has demonstrated antimicrobial activity against Gram-positive bacteria Kiran (2009).

Table: 2Anti-bacterial activity

S.No	Sample (N11)	Zone of inhibition (mm)				
		Salmonella typhi	***E. coli***	***K. pneumoniae***	***Staphylococcus aureus.***	***Bacillus cereus***
1	20 µl	NZ	8	NZ	NZ	NZ
2	40 µl	11	11	08	09	NZ
3	60µl	15	15	12	11	08
4	80	19	17	15	14	11

NZ – No zone of inhibition

CONCLUSION

The isolated novel marine bacterium *Streptomyces* sp simplex in the present study showed a significant response to all the cheaper substrates utilized; in particular, the maximum production was observed with the sunflower oil cake which evidenced its most economical cheaper production. The purified lipopeptide surfactant exhibited a different molecular mass than the earlier reports which constituted it as a pharmaceutical application. Furthermore, an interesting observation on enhanced and consistent crude oil recovering efficiency at wide range of salinity parameters proved that it could be an ideal product for the petroleum industries as well as environmental applications even at hypersaline environmental conditions.

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