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Isolation and identification of *Actinomycetes* with biosurfactant activity

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Abstract:

A total of forty soil samples were collected from different places in Hilla city. Twelve actinomycetes were diagnosed. The ability for producing of biosurfactant was studied ,and found five isolates were able to producing biosurfactant. Actinomycetes spp.8 showed more activity by producing high inhibition on blood agar. Actinomycetes spp.8 was studied and diagnosed as *Streptomyces* spp. These isolate was produce grey aerial mycelium and yellow-green substrate when cultured on yeast –malt extract agar. The biosurfactant material was isolated and the affectivity of biosurfactant on pathogenic bacteria was studied. The biosurfactant extract was effective against *S.aureus* with 14 mm inhibition zone and *E.coli* 10mm inhibition zone and effective against *C.albicans* with 8mm

Introduction:

Biosurfactants are amphiphilic compounds produced by microorganisms as secondary metabolite. The unique properties of biosurfactants make them possible to replace or to be added to synthetic surfactants which are mainly used in food, cosmetics and pharmaceutical industries and in environmental applications (Hamzah, *et al*., 2013).

They are consisting from two parts, a polar (hydrophilic) moiety and a non-polar (hydrophobic) group. The hydrophilic group consists of mono-, oligo-, or polysaccharides, peptides or proteins while the hydrophobic moiety usually contains saturated, unsaturated and hydroxylated fatty acids or fatty alcohols (Pacwa-Plociniczak *et al*., 2011).It is a diverse group of biomolecules, which share the same properties as synthetic surfactant, and in some cases, they are superior in creating water-in-oil or oil in water emulsions (Ashtaputre and shah, 1995; Jacobucci *et al.*, 2009). Several of these biosurfactants are well described chemically ad categorized into high- and low-molecular-mass compounds. The low-molecular- mass biosurfactants include glycolipids and lipopeptides, such as rhamnolipids and surfactin. The high-molecular mass compounds include proteins and lipoproteins, or complex mixtures of these polymers (Desai and Banat 1997, Kimetal 2000, De-souza *et al.*, 2003, Kumar *et al.*, 2006).Microbial derived surfactants have special advantages over their chemically manufactured counterparts because of their lower toxicity, biodegradable nature and effectiveness at extreme

temperature and pH values (Jacobucci *et al.*, 2009).

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces, or excreted extracellularly and contain hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, which are reducing surface and interfacial tension at the surface and interface respectively. They are structurally diverse group of surface active molecules synthesized by microorganisms (Muthusamy *et al*., 2008).Actinomycetes are gram-positive bacteria showing a filamentous growth like fungi. They are aerobic and widely spread in nature (Lo *et al*., 2002).It have ability to producing for producing secondary metabolite such as antibiotic antifungal and antitumor compound ((Deepika, *et al.*,2009,Atta, *et al*., 2012, Zheng *et al*., 2012, Lucas *et al*., 2013).A biosurfactant producing *Streptomyces* spp. was isolated from soil samples collected at the Ennore saltpan, Tamil Nadu, India and the Ennore coast of Bay of Bengal is situated 24 Kms north of Chennai, India (Deepika *et al*., 2010).

This study was aimed to isolation of Actinomycetes isolates with biosurfactant activity and partial extraction of product and effects of against pathogenic microbes.

Materials and Methods:

Sample collection:

Forty soil samples were collected from Hilla city during the period from January 2013 to February 2013.

Isolation of *Actinomycetes* :

Soil samples were serially diluted up to 10^{-6} dilution using sterilized distil water. From each dilution, 1 ml was plated on Starch Casein agar by pour plate. The plates were incubated at 30ºC for 7-10 days (Collins *et al*., 1995).

Characteristics of *Actinomycetes* isolates:

Cultural characteristics of *Actinomycetes* spp. were recorded on YMD (yeast-malt dextrose) agar which includes color of aerial mycelium, color of substrate mycelium and pigmentation actinomycete isolates Shirling and Gottlieb (1966).The morphological characteristics of actinomycete isolates were examined by slide culture method (Williams and Cross 1971, Bergey's 2000). Utilization of carbon sources by the isolate was tested .The ability of actinomycetes of melanin pigments was tested (Shirling and Gottlieb ,1966).

Biosurfactant assay:

Biosurfactant activity of the isolates was evaluated by detection of hemolytic activity. Haemolytic activity was tested using blood agar plate. Blood agar was prepared with human blood (5%) and blood agar base. The blood agar base was sterilized by autoclaving at 121ºC at 15lbs pressure for 15 min. Prior to pouring blood was added and allowed to solidify. The isolates were streaked on the blood agar and the plates were incubated at 28ºC for 7 days. The plates were then observed for zone of clearance around the colonies (Carillo *et al* 1996).

Purification of biosurfuctant:

The culture broth of *Actinomyctes* spp.8 was centrifuged at 10000 rpm for 10 min to remove cells and other degradation metabolites and thereafter clarified with

Millipore membrane filter. The clear sterile supernatant served as the source of crude biosurfactant. The biosurfactant was extracted by liquid-liquid extraction from the cell-free supernatant acidified with 1 N HCl to pH 2.0. The supernatant fluid was mixed with an equal volume of chloroform:methanol (2:1) mixture. The organic phase containing the biosurfactant was collected. The aqueous phase was extracted five more times. The organic extracts were later pooled and dried in a rotary evaporator (Jain *et al.* 1991, 1992).

Antimicrobial activity of crude extract of biosurfactant :

The crude extract were screened for antibacterial activity against *Stapylococous aureus E.coli* and *Candida albicans* by well diffusion method. 100 µl of the crude was placed in wells made on Muller Hinton agar plates seeded with the test bacterial pathogen cultures. The plates were incubated at 37°C and observed for inhibition zone after 24 h. (NCCLS) (2003).

Results and Discussion

Isolation of actinomycetes:

Twelve isolates of actinomycetes were isolated from forty soil samples. These isolates were examined under light microscope and cultural characteristics found that these isolates were belong to actinomycetes spp..These isolates were gram positive, having aerial mycelium, and these isolates were grown on yeast-malt extract agar and the colonies was not raised on agar with earthy odor, and the presence of spore chain.

Screening of actinomycetes isolates for biosurfuctant production:

Five isolates were showed biosufactant activity .The biosurfactant activity was tested on blood agar supplied by red blood cell. All isolates were showed ß-hemolysis for red blood cell by production of inhibition zone (Table 1).

Our results agreed with results obtained by Deepika,*et al* (2010) who found that the biosurfactant production was confirmed by conventional screening methods, including hemolytic, drop collapsing .Blood agar lysis was used for screen of biosurfactant production (Yonebayashi *et al.*,2000; Youssef *et al.*, 2004).

Carrillo *et al*. (1996) was proved the efficiency of this method in screening of biosurfactant-producing bacteria. They found an association between hemolytic activity and surfactant production. Other results found that the blood hemolysis technique is a successes method for the screening of biosurfactant-producing microbes(Bodour and Miller-Maier, 2002).

The *Actinomycetes* spp.(8) was selected for study of biosurfactant activity.

Table (1): Production of biosurfuctant by actinomycetes spp isolates (lysis of blood cells)

Actinomycetes isolates	Inhibition zone (mm)

Actinomycetes spp .1	15
Actinomycetes spp .2	0
Actinomycetes spp .3	10
Actinomycetes spp .4	0
Actinomycetes spp .5	0
Actinomycetes spp .6	0
Actinomycetes spp .7	0
Actinomycetes spp .8	25
Actinomycetes spp .9	0
Actinomycetes spp .10	0
Actinomycetes spp .11	18
Actinomycetes spp .12	23

Cultural characteristic for *Actinomycetes* spp.8:

The cultural characteristics for *Actinomycetes* spp.(8) was studied and the results showed that it was positive for gram stain and having grey aerial mycelium when grown on yeast malt extract agar. These isolate was not able for producing of melanin and other pigments on tyrosine broth medium. It was diagnosed as *Streptomyces* according to morphological and sugar fermentation (Table2).

Table (2):Morphological and biochemical test of *Actinomycetes* spp. 8

***Actinomycetes* spp. 8**	**character**
Gram stain	+
Color of aerial mycelium	grey
Substrate mycelium	Yellow-green
Pigment production	-
Melanin production	-
Sugars fermentation Xylose Mannitol Sucrose glucose	 – + + +

Antimicrobial activity of crude extract of biosurfactant produced by *Actinomycetes* spp .8

Antimicrobial activity of boisurfactant was tested by well diffusion method *Actinomycetes* spp .8 was showed highest activity against *S.aureus* with inhibition zone (14mm) and 10mm against *E.coli* while the results showed that the inhibition zone caused by biosurfactant of *Actinomycetes* spp .8 against *C.albicans* was 8mm (Figure 1). Our results greed with results obtained by Singh, *et. al*, (2007) Who found that the surfactants produced by certain species of bacteria and yeasts exhibit effective antimicrobial activity. Biosurfactants demonstrate antibacterial and antifungal activities,

suggesting possible roles in the medical and agricultural fields (Gunther *et al*., 2005).Surface was found to exhibit effective characteristics like antibacterial, antiviral, antifungal, antimycoplasma and hemolytic activities (Pooja Singh, and S.S. Cameotra 2004).

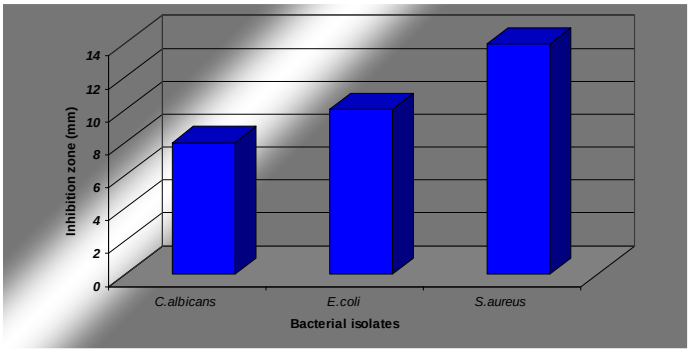

Figure (1): Antimicrobial activity of biosurfuctant produced by *Actinomycetes* spp.8

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الخلاصة:

جمعت حوالي 40 نموذج تربة من مناطق مختلفة في مدينة الحلة وشخصت حوالي 12 عزلة اكتينومايستات .تم فحص قابليتها على انتاج البايوسرفكتنت حيث وجد 5عزلات فقط ها القدرة على انتاج البايوسرفكتنت وكانت العزلة رقم 8 هي فعالة جدا حيث أنتجت اكبر قطر للبايوسرفكتنت على وسط اكار الدم. تم دراسة خصائص هدة العزلة بعد تشخيصها كستربتومايسس حيث وجد إن هده العزلة ذات مايسليم رصاصي ومادة اساس صفراء-خضراء على وسط مستخلص الخميرة والشعير.

وبعدها تم عزل البايوسرفكتنت وتم دراسة تاثيره على البكتريا الممرضة حيث اثبت ان له تاثير على الستافلوكوكس مع قطر تثبيط 14 mm وايضا كان له دور في تثبيط نمو ايكولاي حيث له كان قطر التثبيط 10 mm وقد اظهرت قطر تثبيط 8 mm ضد فطر الكانديدا.