ISOLATION OF NON-PATHOGENIC BIOSURFACTANT PRODUCING BACTERIA FROM KARACHI COAST OF ARABIAN SEA

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ABSTRACT

Biosurfactants are non-toxic, biodegradable surface-active compounds produced by microorganisms. These natural surfactants serve as substitute for chemical surfactants that are toxic and hazardous to the environment. Biosurfactants are gaining much interest because of their industrial and economical value but due to their surface activities biosurfactant producing bacteria can be pathogenic in nature. This study is based on isolation, purification and screening of non-pathogenic biosurfactant producing bacteria from Karachi coast of Arabian Sea. Out of 89 isolates, strain no DGHE2, DGHE6, DGHE10, DGHE12, DGHE32, DGHE52, DGHE55, DGHE56, DGHE62, DGHE71, DGHE85, DGHE86 and DGHE87 were found to be negative for hemolytic activity and showed above 30% emulsification index for at least one of the tested hydrocarbons. The same strains gave high values for BATH assay with hexane and xylene. In the present study we have successfully isolated 13 non-pathogenic bacterial strains capable of producing biosurfactant. Production of such eco-friendly strains would benefit the industries and environment to great extent in the production of biosurfactants.

Key-words: Biosurfactants, non-pathogenic bacteria, hazards, hydrocarbons, industrial applications.

INTRODUCTION

Surfactants mostly used in the industries, are based on chemicals so they are known as chemical or synthetic surfactants. These synthetic molecules are highly toxic to living organisms and are hazardous to the environment. Biosurfactants are amphiphilic surface active compounds produced by microorganisms including bacteria, fungi and yeast (Uzoigwe *et al.*, 2014). They are non-hazardous and environment friendly. Additionally, these compounds can be produced by renewable resources, are low in toxicity, effective in biodegradation and bio-emulsification, possess antimicrobial and anti-cancer activity. These natural surfactants are an alternative to synthetic surfactants and are relatively much valuable because of their economically and industrially significant applications, mainly hydrocarbon bioremediation. Biosurfactants can also be used as detergents and are known significant component in industries like pharmaceuticals, cosmetics, food, beverages and many others (Elazzazy *et al.*, 2015).

Because of surface activity biosurfactant producing bacteria can be pathogenic to humans, plants and animals (Walter *et al.*, 2010). Such pathogenic bacteria cause diseases and life threatening infections by invading, colonizing and growing in the host (Berg *et al.* 2005). They release virulence factors such as toxins in the host cells and evoke diseases (Peterson 1996).

In recent years due to the health and environmental hazards, production of non-pathogenic biosurfactant producing bacteria is gaining much attention. Our present study is based on isolation, purification and screening of non-pathogenic biosurfactant producing bacterial strains from the Karachi cost of Arabian Sea. Bacterial strains are screened on the basis of hemolytic activity to check out the pathogenic strains, emulsification test with 2 hydrocarbons xylene and hexane used to estimate the productivity of emulsifier and BATH assay which is recommended, to determine hydrophobicity of the cell surface.

MATERIALS AND METHODS

SAMPLE COLLECTION

Fourteen samples of marine water were collected from Arabian Sea cost of Karachi. Screening of purified strains was done on the basis of hemolytic activity, emulsification index test and BATH assay.

ISOLATION OF BACTERIAL STRAINS

100 µL of the collected sea water samples were spread over R2A medium and incubated aerobically for 24-48 hours at 37°C (Anandaraj and Thivakaran, 2010). Isolated colonies were then inoculated in Luria Bertani (LB) Broth

(Sambrook *et al.,* 1989). 89 morphologically distinctive colonies were purified through progressive sub-culturing for further screening. Detailed morphological and characterization studies were done and reported as earlier (Shoeb *et al.* 2015).

HEMOLYTIC ACTIVITY TEST

Hemolysis test is the first screening test applied for the identification and isolation of biosurfactant producing bacteria (Carrillo *et al.,* 1996). To check for hemolytic activity the isolates were streaked on blood agar medium (Walter *et al.,* 2010) and incubated for 24-48 hours at 37 °C. After incubation the plates were then visualized for clear zones of hemolysis around colonies which would indicate biosurfactant production.

EMULSIFICATION INDEX TEST (E24)

Emulsification index (E24) was tested for the isolates for 2 hydrocarbons hexane and xylene. 1.5mL of hydrocarbon was mixed with 1.5mL of cell free broth and kept to rest for 24hours. The emulsification index percentage was then calculated as reported by (Asfora *et al.,* 2006*)* using formula:

E24 = Height of emulsion formed x 100/total height of solution.

BATH ASSAY

Cell hydrophobicity was estimated using BATH assay According to the protocol given by Rosenberg *et al.* (1980). Bacterial cells were washed and suspended in salt buffer containing 16.9g/lit of K_2HPO_4 and 7.3g/L of KH_2PO_4. Optical density (O.D.) was measured at 600nm. 2mL of cell suspension was mixed for 3min with 100µL of hydrocarbons hexane and xylene. After vortex mixing, the percentage of cell adherence to hydrocarbons was measured using equation:

1 – (OD of the aqueous phase/OD of initial cell suspension) × 100.

RESULTS

ISOLATION OF BACTERIAL STRAINS

Total of 89 bacterial colonies were isolated, purified and screened for the biosurfactant production. The isolated strains were coded as DGHE01-89. Detailed results, regarding characterization morphological and screening tests for biosurfactant production are reported in our previous studies (Shoeb *et al.,* 2015).

HEMOLYTIC ACTIVITY TEST

Purified strains were streaked on blood agar plates containing 5% (v/v) Human blood and incubated at room temperature for 24 h to inspect the hemolytic activity of strains. Out of 89 isolates, 24 Strains showing negative results were selected for further studies as shown in Table 1.

EMULSIFICATION INDEX TEST (E24)

Out of the 24 non-hemolytic isolates selected for evaluating the emulsification capacity, 13 strains showed positive emulsification Index (E24) of above 30% with at least one of the two hydrocarbons tested, shown in Table 1.

BATH ASSAY

Cell surface hydrophobicity of non-hemolytic strains was estimated through BATH assay. Affinity of cell adherence was shown in the range of 5%-86% for hexane followed by 1%- 99% for xylene as shown in Table 1. Strain DGHE85 showed maximum cell adherence with hexane while strain DGHE87 gave best results with xylene.

DISCUSSION

Biosurfactants are amphiphilic surface active microbial molecules that can reduce surface and interfacial tension in hydrocarbon mixtures as well as aqueous solutions (Bento *et al.*, 2005). These active agents are capable of emulsifying oil in water and can assist in biodegradation of compounds (Shoeb *et al.,* 2015). Biosurfactants have been most promising because of their versatile industrial application, high productivity from renewable resources and biochemical properties. Most of all these natural surfactants are environment friendly, low in toxicity and effective even at extreme conditions (Uzoigwe *et al.,* 2014). They can be used in herbicide and pesticide formulations, pharmaceuticals, cosmetics, as foaming agents, detergents, soaps and many other practically essential supplies (Elazzazy *et al.,* 2015).

Blood agar lysis method is commonly used to screen for biosurfactant production (Banat 1993). Mulligan *et al.* (1984) recommended blood agar lysis method as a preliminary screening method, whereas, Yonebayashi *et al.* (2000) recommended it as the sole method for the screening of biosurfactant producing bacteria. However, very few studies have mentioned the possibility of biosurfactant production in bacteria without a hemolytic activity (Schulz *et al.*, 1991). Strains that show hemolysis of red blood cells are believed to be the producer of virulence factors that can lyse the blood cells. Such bacterial strains are considered as pathogenic, since they can effectively invade the host tissues and organs, colonize and proliferate ultimately leading to diseases and lethal infections to the host (Berg *et al.*, 2005). Because of health and environmental hazards many concerns are being raised regarding pathogenicity of biosurfactant producing bacteria and utilization of such bacteria for the production of biosurfactants at industrial level.

In the present investigations we selected strains that showed negative hemolytic activity and conducted screening tests for biosurfactant production. We successfully isolated 13 bacterial strains (Table 1) that are negative for hemolytic activity and still producing biosurfactant evident through emulsification and their adherence potential to hydrocarbons. Emulsification Index test is considered as one of the important screening tests to support the selection of potential biosurfactant producers and it is assumed that if the cell free culture broth used in this assay contains biosurfactant then it will emulsify the hydrocarbons present in the test solution (Satpute *et al.*, 2008). Emulsification index test with more than 30% is considered as significant value for the screening of biosurfactant producing bacteria (Joshi and Shekhawat, 2014). Another screening test conducted was BATH assay. In this test cell adherence with hydrophobic compounds is measured as cells attach themselves with oil droplets by producing biosurfactants, which is considered as a method to screen bacteria for biosurfactant production (Franzetti *et al.*, 2009). Strains showed significant emulsification activity along with positive BATH assay results.

Table 1. Biosurfactant production activity of non-pathogenic bacteria isolated from the Karachi coast of Arabian Sea.

S. No.	Strains Code	E24 with Hexane (%)	E24 with Xylene (%)	BATH assay with Hexane (%)	BATH assay with Xylene (%)
1.	DGHE02	32	24	40	33
2.	DGHE06	4	40	5	23
3.	DGHE10	4	36	12	29
4.	DGHE12	36	24	28	15
5.	DGHE32	32	32	43	29
6.	DGHE52	36	04	37	29
7.	DGHE55	36	28	25	24
8.	DGHE56	36	16	24	59
9.	DGHE62	32	36	34	60
10.	DGHE71	32	32	26	01
11.	DGHE85	32	36	86	70
12.	DGHE86	40	36	73	66
13.	DGHE87	32	08	57	99

As shown in Table 1 strains DGHE 2, 6, 10, 12, 32, 52, 55, 56, 62, 71, 85, 86 and 87 were found to be negative for hemolytic activity and showed above 30% emulsification index for at least one of the two hydrocarbons hexane and xylene. The same strains gave high values for BATH assay with hydrocarbons hexane and xylene. Hence these strains isolated from the Karachi coast of Arabian Sea are proved to be non-pathogenic biosurfactant producers. In the future such bacterial strains can be utilized for mass production of biosurfactant at industrial level.

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