IJASRM
International Journal of Advanced Scientific Research and Management, Volume 3 Issue 7, July 2018
www.ijasrm.com
ISSN 2455-6378

Molecular Detection of Rhlb Gene from Biosurfactant Producing Pseudomonas Aeruginosa Isolated From Oil Contaminated Soil

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Abstract

Biosurfactants are the compound which helps in reducing surface tension. These compounds help in stabilizing emulsions, promoting foaming, and reducing surface tension etc. A total of 50 samples were collected from different soils contaminated with oil in Chennai area. 32/50(64%) of Pseudomonas aeruginosa were isolated from oil contaminated soil samples. The surface tension of the medium was found ranging from 0.020 to 0.071N/m-1. Among 32 Pseudomonas aeruginosa isolates, 21/32(66%) of them reduced the surface tension. All the 32 isolates of Pseudomonas aeruginosa produced beta hemolytic colonies on blood agar which includes the biosurfactant producing strains. All the 21 biosurfactant producing Pseudomonas aeruginosa isolates showed good antagonistic activity against S.aureus, Bacillus subtilis and showed less activity with E.coli. The biosurfactant producing Pseudomonas aeruginosa produced a clear zone (2.8mm) in oil spreading assay. As an outcome of our work, E24 was found to be highest for petrol with 71% followed by kerosene (63%) and diesel (57%).rhlB gene was detected in 21 isolates, which was about 226bp in size.

Keywords: *Biosurfactant, Pseudomonas aeruginosa, Emulsification test, rhlB gene.*

1. Introduction

Biosurfactants are amphipathic (Lang, 2002) surface active molecules produced extracellular by various bacteria, fungi and yeasts (Arimaet al., 1968). They can reduce surface and interfacial tension which makes them potential candidates in enhancing oil recovery and bio emulsification processes (Lin, 1996). Now a days, increased use of petroleum hydrocarbons and oils as main energy sources has increased the risk of leakage of these products while transportation, leading to soil and water contamination; which is not beneficial for human beings, plants, animals and microbes.

Biosurfactant producing microorganisms are naturally present in the oil contaminated soil. Oil contaminated environment contain large amount of hydrocarbons (Suganya, 2013).Bacteria produces biosurfactants which helps them in emulsifying the hydrocarbons and utilize them as substrate by mineralizing them converting them into harmless product.

Rhamnolipid and surfactin are the important classes of biosurfactants which have been widely studied. The most commonly isolated biosurfactants are glycolipids and lipopeptides. They include rhamnolipids released by Pseudomonasaeruginosa (Nitschkeet al., 2005), sophorolipids from Candida sp.(Daverey, &Pakshirajan, 2009), as well as surfactin produced by Bacillus subtilis strains(Ahimouet al.,2000). This study was therefore taken up to screen biosurfactant producing Pseudomonas aeruginosa from oil contaminated soils and molecular detection of rhlB gene.

3. Materials and Methods

a)Sample collection:

We collected 50 soil samples from different oil contaminated areas in Chennai. They were obtained

from a depth of 2-3 inches from the ground level and collected in a sterile polythene bag and were taken to the laboratory and processed.

b)Processing of the sample:

one gram of each soil sample was added into 50 mL of minimal salt medium(Tahzibiet al., 2004) containing (g/L); 15 g NaNO3, 1.1 g KCl, 1.1 g NaCl, 0.00028 g FeSO4.7H2O,3.4 g KH2PO4, 4.4 g K2HPO4, 0.5 g MgSO4.7H2O, 0.5 g yeast extract at 37°C in shaker incubator (100 rpm). After 48 h of incubation, the sample was serially diluted using sterile saline (0.85% NaCl) and Pseudomonas aeruginosa was selected based on the colony morphology, gram staining and pigment production on nutrient agar. Pseudomonas aeruginosa was identified as per the standard biochemical methods. The selected isolates were screened for the production of biosurfactants using following screening methods.

c)Screening for the production of Biosurfactant

Screening for the production of biosurfactant in Pseudomonas aeruginosa was done by the following methods-Surface tension, Antagonistic test, Haemolytic test and Emulsifying test.

i) Surface tension:

Based on the surface tension reducing capacity of Pseudomonas aeruginosa on oils was used to screen the bio surfactants and it was calculated by using standard drop weight method (Makkar, &Cameotra,1997, Falatko, 1991).

Mass of one drop of the medium,

m = W2-W1 / total droplet.

Where,

W2 : weight of the sample with beaker.

W1 : weight of the empty beaker.

ii) Haemolytic test:

Pure culture of bacterial isolates were streaked on the freshly prepared blood agar and incubated at 37°C for 48-72 h. Results were recorded based on the hemolytic pattern (Mulliganet al., 1989).

iii) Antagonistic test:

The antagonistic property of Pseudomonas aeruginosa was tested with Bacillus subtilis, Staphylococcus aureus and Escherichia coli, for the antimicrobial activity of the bio surfactant produced in the medium (Abaloset al., 2001).

d) Oil Spreading Assay:

10 µl of crude oil was added to the surface of 30 ml of distilled water in a petridish to form a thin oil layer. 10 µl of culture or culture supernatant was gently placed on the centre of the oil layer. If biosurfactant was found in the supernatant, the oil was displaced and a clearing zone was formed. The diameter of this clearing zone on the oil surface correlates to surfactant activity, also called oil displacement activity (Jain et al., 1991).

e) Emulsification test (E24):

Several colonies of pure culture were suspended in test tubes containing 2 ml of mineral salt medium. After 48 h of incubation, 2 ml hydrocarbon (oil) was added to each tube. Then, the mixture was vortexes at high speed for 1 min and allowed to stand for 24 h. The emulsion index (E24) is the height of the emulsion layer (cm) divided by total height (cm), multiplied by 100 (Bodouret al., 2004).

$$\textit{Emulsification index}(e24) = \frac{HEL}{\textit{total height}} X100$$

Where

HEL - Height of the emulsion layer

Molecular detection of rhlBgene from Pseudomonas aeruginosa:1.5 ml of overnight broth cultures were taken in micro centrifuge tubes to isolate the genomic DNA by using modified method of Ochsneret al.,(1994).The isolated DNA samples were run in anagarose gel electrophoresis there the dark orange bands were observed under UV trans-illuminater and isolated samples were subjected to PCR analysis to detect the rhlBgene with the help of kpd1 (forward primer:5_ GCC CAC GAC CAG TTC GAC 3_) andkpd2 (reverse primer: 5_ CAT CCC CCTCCC TAT GAC 3_) primers which has homologous sequence with 1030-1048 bp region of the rhlB gene. Amplification was done with the following PCR cycling conditions: initial denaturation:94oC, 2mints, de-naturation: 94oC, 15sec, annealing:54oC, 15sec, extension: 72oC, 15sec, final extension: 72oC, 2mints. The final PCR product was ran in 1% agarose in TE buffer and the gel was stained with ethidium bromide. After the completion of reaction, add 5µl of loading dye, mix it and load the total 25µl into agarose gel electrophoresis.

4. Results and Discussion

A total of 50 soil samples were collected from different oil contaminated areas in Chennai.

32/50(64%) of Pseudomonas aeruginosa were isolated from oil contaminated soil samples. The viable cells ranging from 1.9x106 to 7.3x107 CFU/gm were isolated from oil contaminated soil.

a) Surface tension:

Surface tension was determined and the values were determined and compared with the control media. The surface tension of the medium was found ranging from 0.020 to 0.071N/m-1. Among 32Pseudomonas aeruginosa isolates, 21/32(66%) of them reduced the surface tension.

b) Haemolytic test:

In general all the 32 isolates of Pseudomonas aeruginosa produced beta hemolytic colonies on blood agar which includes the biosurfactant producing strains.

c) Antagonistic test:

This test was done to detect the antagonistic effect of rhamnolipid produced by Pseudomonas aeruginosa in fermented media. All the 21 biosurfactant producing Pseudomonas aeruginosa isolates showed good antagonistic activity against S.aureus, Bacillus subtilis and showed less activity with E.coli.

d) Oil Spreading Assay

Oil displacement tests are indicative of the surface and wetting activities. All the 21 biosurfactant producing Pseudomonas aeruginosa produced a clear zone (2.8mm) in Oil spreading assay.

e) Emulsification test (E24)

All the 21 biosurfactant producing Pseudomonas aeruginosa isolates were tested for their emulsifying activity using kerosene, petrol and diesel. Of all the three different oils tested E24 was found to be highest for petrol with 71% followed by kerosene (63%) and diesel (57%).

Molecular detection of rhlBgene From Pseudomonas aeruginosa:

Molecular detection of rhlB gene was done for all 32 Pseudomonas aeruginosa isolates. rhlBgene was detected in 21 isolates, which was about 226bp in size. The isolates which harboured rhlB gene were positive biosurfactant producers.

Surfactants are usually organic compounds containing both hydrophobic group and hydrophilic group (Pornsunthorntaweeet al., 2008). Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Bio-surfactants are surface active substances synthesized by living cells; they are generally nontoxic and biodegradable. Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions, and potential applications in environmental protection (Banat et al., 2000 &Youssefet al., 2004).

In our study, a total of 50 samples were collected from different oil contaminated areas in Chennai. 32/50(64%) of Pseudomonas aeruginosa were isolated from oil contaminated soil samples. The viable cells ranging from 1.9x106 to 7.3x107 CFU/gm were isolated from oil contaminated soil. This was found to be in agreement with that of Anandarajand Thivakaran, (2010).

The surface tension of the medium was found ranging from 0.023 to 0.069N/m-1. Among 32 Pseudomonas aeruginosa isolates, 21/32(66%) of them reduced the surface tension. All the 32 isolates of Pseudomonas aeruginosa produced beta hemolytic colonies on blood agar which includes the biosurfactant producing strains. This was found to be in agreement with that of Branch (2012).All the 21 biosurfactant producing Pseudomonas aeruginosa isolates showed good antagonistic activity against S.aureus,Bacillus subtilis and showed less activity with E.coli. The biosurfactant producing Pseudomonas aeruginosa produced a clear zone (2.8mm) in oil spreading assay. This was found to be in consistent with that of Chandran & Das, (2010). E24 was found to be highest for petrol with 71% followed by kerosene (63%) and diesel (57%). This was found to be in agreement with that of Okerentugba, & Ezeronye, (2003).

Molecular detection of rhlB gene was done for all 32 Pseudomonas aeruginosa isolates. rhlBgene was detected in 21 isolates, which was about 226bp in size. The isolates which harboured rhlB gene were positive biosurfactant producers. This was found to be in agreement with Mathiyazhaganet al., (2011).

5. Conclusion

Pseudomonas aeruginosa isolated from oil contaminated soil showed biosurfactant producing ability. rhlBgene was detected in 21 isolates, which was about 226bp in size. Biosurfactant are used in oil industry, foods, cosmetics, pharmacology and environmental technology because of their ability to

stabilize emulsions. The features that make them commercially promising alternatives to chemically synthesized surfactants are their lower toxicity, higher biodegradability and greater environmental compatibility.

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