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Research Article

ISOLATION AND IDENTIFICATION OF BIOSURFACTANT PRODUCING BACTERIA FROM OIL SPILLED SOIL

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ABSTRACT

The study focused on the isolation and identification of bacterial strain from crude oil contaminated soil. 16 bacterial colonies (S1- S16) were isolated using Bushnell Hass medium supplemented with hydrocarbon and screened for biosurfactant analysis. Among these S3 culture showed the positive result using preliminary tests. The strain was isolated and identified by 16s rRNA gene sequence analysis as *Rhodococcus erythropolis*. With the above findings, the isolated strain has good oil degrading capacity and can be potentially applied in bioremediation for conservation of the soil and ecosystem.

INTRODUCTION

The release of crude oil into the environment is the potential risks posed to the ecosystem. Crude oil is a complex hydrocarbon, composed of hydrogen and carbon atoms. Oil based Pollution of the environment causes major impact to plants, animals and human beings. Bacterial degradation of hydrocarbons has greater importance to degrade a host of recalcitrant compounds. The ability of bacteria to degrade hydrocarbons is a viable alternative to clean up the environment. One of these degradative pathways is through the production of biosurfactants, exhibited greater advantages than their chemical degradation.

MATERIALS AND METHODS

Isolation and enrichment of biosurfactant producing bacteria

Biosurfactant producing bacterial strain was isolated and enriched from automobile workshops oil contaminated soil in Sivakasi, TamilNadu, India using nutrient agar medium by serial dilution method.

Preliminary test for biosurfactant production

Screening of microbial biosurfactant

The selected bacterial isolates were grown in Bushnell Hass medium supplemented with petrol (1%) as carbon source at 37^0C in shaking incubator with 150 rpm for 24hrs. The supernatant of bacterial isolate was collected using centrifugation at 5000 rpm for 15 min in cooling centrifuge.

Drop collapse test

The supernatant of the bacterial cultures (24 hrs old) were taken in a clean glass slide. Uninoculated BH medium used as a control. About 25µl of cell free supernatants were pipette out as droplet on paraffin coated glass slide, collapse of drop was recorded within a minute (Kuiper *et al*., 2004).

Oil spreading test

20 ml of distilled water was added to a Petri dish to this 20 µl of crude oil was added to the surface of water followed by 10 µl of culture extract from the bacterial isolates were dropped on to the oil surface. The diameter of clear zones on the oil surface was measured and recorded. A negative control was

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maintained with distilled water (without surfactant), in which no oil displacement or clear zone (Morikawa *et al*., 2000).

Quantitative determination of biosurfactant activities

The selected isolates were grown in BH medium for 24h at room temperature. The culture supernatant was centrifuged at 5000 rpm for 20 min and cell free supernatants were used for the tests. 2 ml of cell free broth was mixed with 2 ml of hydrocarbon in a test tube. It was then vortexed for a minute using a cyclone mixer and left undisturbed for 24 h (Kim *et al*., 2004). After incubation, the height of emulsified layer was measured and emulsification index (E24) was calculated using the formula,

$$E24 = \frac{\text{Height of emulsified layer (cm)}}{\text{Total height of liquid column (cm)}} \times 100$$

Morphological, Colonial and Biochemical characterization of bacterial Isolate

The isolate was identified according to Gram staining, colonial characteristics and biochemical characterization (Rakesh and Kiran, 2004).

Identification of Biosurfactant producing bacteria by 16S rRNA gene sequencing

Genomic DNA of the strain was isolated by the phenol chloroform extraction method. Bacterial isolate exhibiting high biosurfactant producing capability was selected and identified by partial 16S r RNA sequencing. The nucleotide sequences obtained was compared with sequences of GenBank using nBLAST search. The phylogenetic tree was constructed by using MEGA 6 software version (Tamura *et al*., 2011).

RESULTS AND DISCUSSIONS

Isolation and enrichment of biosurfactant producing bacteria

In this study 16 bacterial strains (S1-S16) were collected from 2 different petroleum hydrocarbon contaminated soil samples by using Bushnell Hass medium with 1% petrol out of the 16 strains, 1 potent strain was selected based on their improved hydrocarbon degrading capacity (Figure 1).

Figure 1 Isolate S3 on Nutrient agar medium

Biosurfactant analysis

Several micro organisms were utilizing petroleum hydrocarbons as their carbon sources are known to produce biosurfactants. In this study, the physiochemical properties of biosurfactants (S3) were characterized by oil displacement, drop collapse and Emulsification index (Table 1). The culture filtrate of S3 collapsed the drop within 10 sec. This implies that the isolate had ability to produce surface active compounds, which reduction in surface tension. The similar result was observed earlier by Peng *et al*., 2007 and Tian *et al*., 2016. Pacheco *et al*., 2010 reported that *Rhodococcus erythropolis* was applicable to remove oil. Likewise S3 exhibit well oil displacement property with respective zone of 15mm. The emulsification index of the S3 was 65% when compared to the chemical biosuurfactant SDS (75%). Emulsification plays a key role in biosurfactant production. It was one of the criteria to support the selection of potential biosurfactant producers (Bonilla *et al*., 2005).

Table 1 Screening of Biosurfactant producing bacteria (S3)

S.No.	Test	Result
1.	Oil spreading method	15mm
2.	Drop collapse	Positive
3..	Emulsification index	65%
	SDS	75%

Identification of bacteria

The bacterial strain was identified by morphological and molecular sequencing. Morphological analysis indicated that the strain was aerobic, non- sporulating and non- pigmented bacteria (Table 2). By Gram's staining the strain was found to be gram–positive cocci. Molecular characterization of S3 was performed by 16s rRNA gene sequencing analysis and the nucleotide sequences of the S3 was deposited in the National Center of Biotechnology information (NCBI Gen Bank) under the respective accession number KY940073 designed as *Rhodococcus erythrobolis* respectively. Phylogenetic tree of S3 were constructed and shown in Figure 2 .The 16s rRNA gene sequence of S3 revealed 97% identity to *Rhodococcus erythrobolis* KY940073. The earlier researcher also demonstrated that *Rhodococcus erythropolis* had ability to produce biosurfactant that play key role to remediate the oil contaminated area (Tian *et al*., 2016).

Table 2 Biochemical characterization of isolate S3

Character	Response	Character	Response
Cell Shape	Fragmented mycelium, non- sporulating	Voges Proskauer test	-
Color of aerial mycelium	Pink	Indole production	-
Gram's staining	Positive	Citrate utilization	-
Pigment production	Negative	Amylase	+
Optimum temperature	30˚C	Caseinase	+
Optimum pH	7	Chitinase	+
Catalase Production	+	Urease	+
Hydrogen Sulfide Production	-	Cellulase	+
Nitrate reduction	-	Arginine hydrolase	-
Starch hydrolysis	+	L- Asparaginase	+
Methyl red test	-	Lipase	+

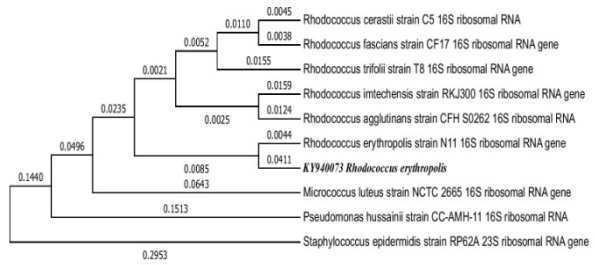

Figure 2 Phylogenetic analysis of 16s rRNA gene sequencing of strain KY940073 and the type strain of closest cocci, using the neighbour – joining method. Horizontal branch lengths are proportional to evolutionary divergence.

CONCLUSION

It is concluded that, the biosurfactant produced by *Rhodococcus erythropolis* was exhibited good hydrocarbon degradation. Hence this strain will be an efficient tool for remediation of crude oil contaminated sites.

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References

Bonilla, M., Olivaro, C., Coronal, M., Vazquez, A. and Soubes, M. 2005. Production and characterization of a new bioemulsifier from *Pseudomonas pudita* ML2. *J. Appl. Microbiol*. 98: 456-463.

Kim, P.I., Bai, H., Bai, D., Chae, H.S., Chun, Y. and Kim, R. 2004. Purification and characterization of a lipopeptide produced by *Bacillus thuringiensis* CMB26. *J. Appl. Microbiol*. 97: 942-949.

Kuiper, I., Lagendijk, E.L., Pickford, R., Derrick, J.P., Lamers, G.E., Thomas-Oates, J.E., Lugtenberg, B.J. and Bloemberg. G.V. 2004. Characterization of two Pseudomonas putida lipopeptide biosurfactants, putisolvin I and II, which inhibit biofilm formation and break down existing biofilms. *Mol. Microbiol.* 51(1): 97-113.

Morikawa, M., Hirata, Y. and Imanaka, T.A. 2000. A study on the structure-function relationship of lipopeptide biosurfactants. *Biochim. Biophys. Acta.* 1488: 211- 218.

Pacheco, G.J., Ciapina, E.M.P., Gomes, E.B. and Junior, N.P. 2010. Biosurfactant Production by *Rhodococcus Erythropolis* and its Application to Oil Removal. *Braz. J. Microbiol.* 41(3): 685-693.

Peng, F., Liu, Z., Wang, L. and Shao, Z. 2007. An oil degrading bacterium: *Rhodococcus erythropolis* strain 3C-9 and its biosurfactants. *J. Appl. Microbiol*. 102: 1603-1611.

Rakesh, P. and Kiran, P. 2004. Identification of bacteria. Experimental Microbiology-I. Third edition Aditya Publication, Ahmedabad, India, pp 95-101.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*. 28: 2731-2739.

Tian, Z., Chen, L., Li, D., Pang, H., Wu, S., Liu, J. and Huang, L. 2016. Characterization of a Biosurfactant-producing Strain *Rhodococcus sp*. HL-6. *Rom. Biotechnol. Lett*. 21(4): 11650-11659.

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