Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (12):71-80
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Isolation of Oil-degrading bacteria from spill samples and studying their biodegradation potentiality on different types of oils

Aniruddha Dey, Saharsh Maheshwari, Anurag, Synchon Mandal and Suneetha V.

School of Biosciences and Technology, VIT University, India

ABSTRACT

The use of bacteria to degrade hydrocarbons from oil-spill sites is one of the most important process of bioremediation of the environment. Oil-spill contaminated samples collected from sewage plants, petrol pumps and harbors were used to get the potential bacterial colonies by pure culture techniques. Different staining techniques were implemented on the isolated bacterial strains for better studying and identification of them. The bacterial colonies so obtained were inoculated in a nutrient broth containing 1ml of oils individually and then mixed oils respectively and the optical density was checked after every 24 hours for 5 days. Finally zone-inhibition test was done to confirm the oil degrading nature of the isolated bacterial colonies.

INTRODUCTION

One of the most hazardous disasters to occur in nature is oil-spills. Oils in general have a tendency to clog the place where it falls-be it soil or water. Oil-spills lead to a huge impact on the environment niche disrupts the biodiversity and leads to a huge change in both the macroenvironment and microenvironment. Thus to bring back the original balance in the environment, the oil needs to be degraded away. This can be done both by chemical and biological means [4]. Biodegradation is a biological process where microbes slowly degrade the oil away and thereby improving the environment [3]. Oil-spills have become a major threat to the maintenance of balance of biodiversity and so recently for a few decades many researches are been carried out in this field to find the best and most effective biodegrading agent for removal of oil from oil contaminated sites. Few of the best oil degrading bacterial strains include those of Bacillus, Pseudomonas, Acinetobacter, Alcanivorax, Marinobacter, etc [1][4][5]. Oil-degrading bacteria utilize oils as one of their metabolites to get the source of carbon and energy thereby reducing it to its corresponding lower forms until it becomes non-hazardous. Thus oil-degrading bacteria provides a clean solution for the removal of oils from oil-spill sites.

Oil-spills cause many imbalances in the environment. Aqueous species are mostly affected by these [2]. Oils tend to coat their body and gills thereby preventing them from respiring properly and thus leading to their death. Even birds near oil-spill sites are affected when they try to catch the fishes. Their feathers become coated with oil, they cannot fly properly. Moreover they try to remove these oils with their beaks leading to ingestion of oils which would affect their body and ultimately leading to their death. Biodegradation of oil is a faster and more effective process of degrading oil from oil-spill sites than other chemical processes. This is because chemical methods are time-consuming and would also give rise to other by-products which have to be further removed [7]. Hence this method is more expensive. On the other hand,bacteria break the hydrocarbon of oils into simpler products like carbon-dioxide and water which are completely harmless [6]. This biodegradation is a safer and cheaper method of removal

of oils from oil-spill sites. This work relates to the isolation and identification of potential oil-degrading bacteria from oil-spill sites and studying their oil degradation potentiality of different types of oils.

MATERIALS AND METHODS

- Nutrient Agar powder
- Bacteriological Agar-Agar powder
- Distilled Water
- Samples from Oil-Spills
- Oil Samples

Fig 1: Some of the ingredients used to make the media

Fig 2: Different types of oils

Fig 3: Samples from oil-spill sites

Fig 4: Collection of oily soil near a gas station

Fig 5: Collection of oily water from a sewage treatment plant

METHODOLOGY

Different soil sample was collected as a natural source for oil degrading bacteria. Variety of oil samples were also collected to analyze the effect of bacteria on different sample.

A process of serial dilution was followed for lowering the inoculum load in which soil samples were used as source of bacteria. Six test tubes were taken with distilled water and the load was reduced up to 10^{-6}dilution.

Nutrient Agar media of 100ml (100 ml of distilled water with 3.6g nutrient agar powder and 2g agar-agar powder), in a conical flask, was prepared and with petri dishes it was put for sterilization. Pour plate technique was implemented inside Laminar Airflow, followed by 48 hours incubation for getting the bacterial colonies from different samples.

Fig 6: Sterilisation of nutrient media and the petri plates

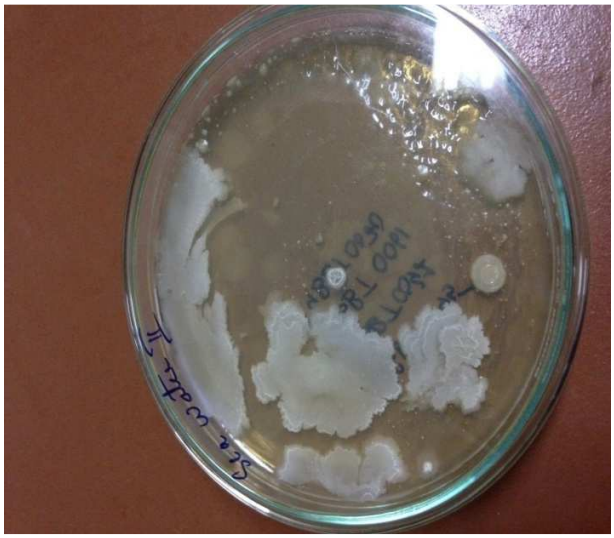

Fig 7: Colonies obtained from sea water along with contaminants

Fig 8: Colonies obtained from oily water along with contaminants

Repeated sub-culturing was performed to keep the colonies fresh. Streaking was done with sterile inoculation loop to get isolated form of bacterial colonies.

Fig 9: Streaking of colony from sea water

Fig 10: Streaking of colony from second sample of sea water

Gram staining was performed on the bacterial colonies to identify the basic morphology of them.

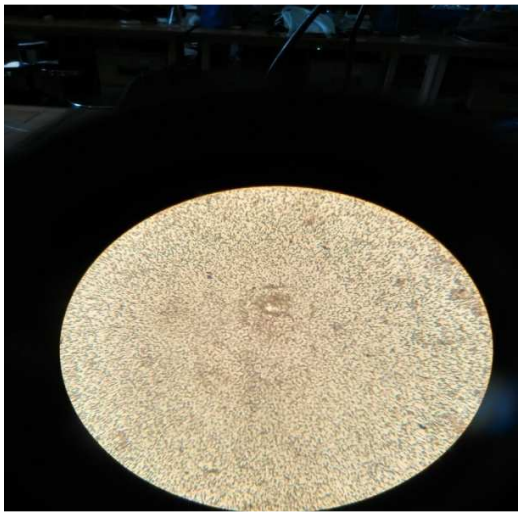

Fig 11: Gram staining of the colony from sea water under 40X magnification

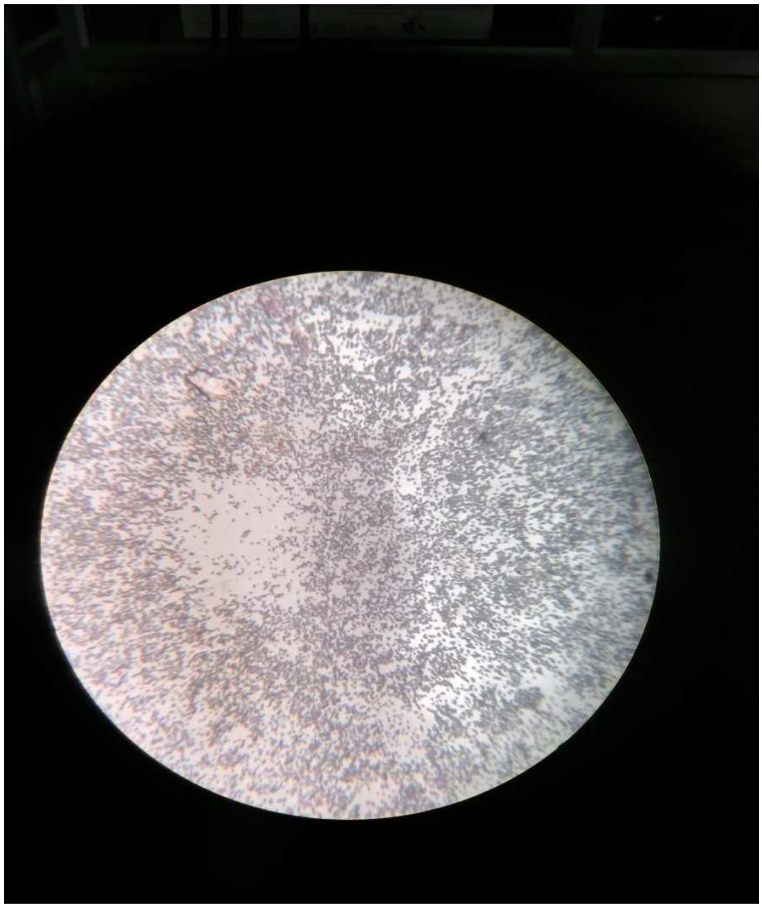

Fig 12: Gram staining of the colony from oily water under 40X magnification

Nutrient broth of 100ml (3.25g nutrient broth powder in 100ml of distilled water) was made in 5 different Side Arm flasks and was sterilized. The bacterial colony was transferred with help of inoculation loop in 4 of the Side Arm flasks leaving one for the control. Different oil samples of 1ml each was poured in different flasks. The flasks were

kept inside Agitator at 150 rpm rate.Optical density reading was taken for next four days to analyze the action of bacteria on different oil samples.

Fig 13: Side arm flasks containing nutrient broth along with oil inoculated with bacterial colonies

Fig 14: OD reading of one of the culture broth

Nutrient broth of 100ml was made in 5 different Side Arm flasks and was sterilized.The bacterial colony was transferred with help of inoculation loop in 4 of the Side Arm flasks leaving one for the control. A mixture of oil samples containing 1ml of each oil sample was transferred to all the flasks. The flasks were kept inside Agitator at 150 rpm rate.Optical density reading was taken for next four days to analyze the action of bacteria from different sources on the mixture of oil samples.

Fig 15: Preparation of broth with mixed oil samples and inoculums in a laminar flow

LIPASE ACTIVITY:

Lipase activity of the bacterial colony was tested. Nutrient agar was prepared in a petri plate aseptically. After the agar solidified a thin layer of mustard oil was poured over the agar gel. Next, a bacterial colony which was obtained (which maybe of oil-degrading type) was taken out by a sterilized loop and put on the oil film. Similarly many colonies were put at specific places on the thin layer of mustard oil. The petri plate was sealed and incubated for 60 hours. After 60 hours degradation of oil was checked in the petri plate.

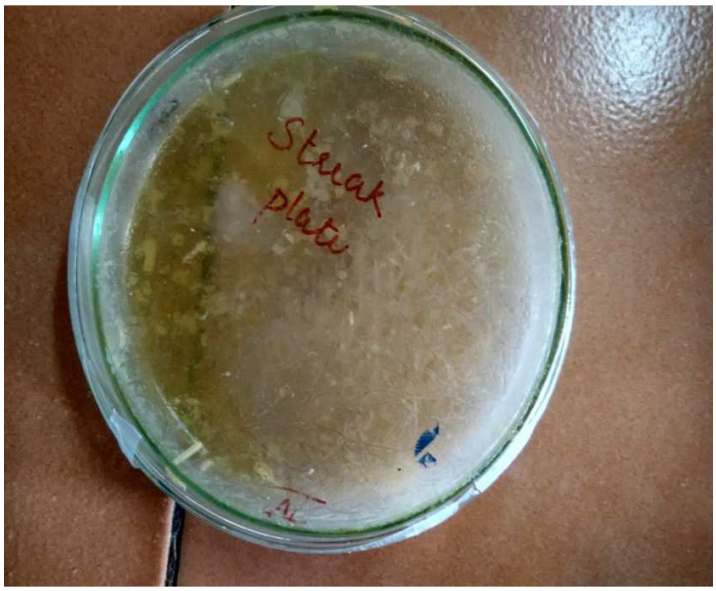

Fig 16:Oil degradation as seen on the petri plate (right side)

RESULTS AND DISCUSSION

A considerable amount of oil-degradation was seen in the petri plate on which lipase-activity was tested. Almost three-fifth of the thin layer of oil was degraded. It can henceforth be said that the bacterial colonies which was obtained from the oil-spill sites were oil-degrading bacteria. This is because when these bacteria were isolated and tested for oil-degradation they showed positive results. It can also be said from the analysis that these bacteria can degrade a variety of oil because these bacteria were mostly isolated from crude oil-spill sites and they showed degradation of even mustard oil. Thus these bacteria can be used to degrade away a multiple type of oils.

Acknowledgement
We want to express our sincere gratitude to our honourable chancellor Dr. G. Viswanathan for his constant support and we want to extend our thanks to our respected vice-presidents Mr. Sankar Viswanathan, Mr. Sekar Viswanathan and Mr. GV Selvam for their motivation to do this research.

REFERENCES

[1]D. Bhattacharya, PM. Sarma, S. Krishnan, S. Mishra, B. Lal, *Appl. Environ. Microbiol.* , **2002**, 69, 1435.
[2]S. Mishra, J. Jyot, RC. Kuhad, B. Lal, *Appl. Environ.Microbiol.*,**2001**, 67, 1675.
[3]P. Morgan, RJ. Watkinson, *"Biodegradation of components of petroleum," In C. Ratledge (ed.)., Biochemistry of microbial degradation*, **1993**.
[4]AR. Johnsen, LY. Wick, L. Harms, *"Principle of microbial PAH-degradation in soil." Environ. Pollut.*, **2005**, 133, 71-84.
[5]WFM. Röling, MG. Milner, DM. Jones, K. Lee, F. Daniel, RJP. Swannell, IM. Head, *"Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation." Appl. Environ.Microbiol.*, **2002**, 68, 5537-5548.
[6]CE. Zobell, *Action of microorganisms on hydrocarbons. Bacteriol.Rev.*, **1946**, 10, 1-49.
[7]R. Margesin, F. Schinner, *Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl. Microbiol. Biotechnol*., **2001**, 56, 650-663.