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Short Communication:

Selective Screening of Potential Crude Oil Degrading Microbes from Crude Oil Contaminated Site

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Abstract

Crude oil contamination is one of the major problems in the current environment pollution. To get rid of this, physical, chemical and biological methods are applied either alone or in combination. Use of microbes is one of the most popular methods among them. In this experiment, soil samples collected from the oil field to isolate potential microbes capable of crude oil degradation. Isolation of the crude oil degrading bacteria was followed by enriching the microbes by providing suitable growth conditions. The microbes those were capable of degrading the crude oil were identified as Bacillus cereus, Pseudoxanthomonas mexicana, Halomonas daqingensis and Parapusillimonas granuli, by 16s rRNA sequencing.

Keywords: Crude oil degradation, microbial degradation, 16s rRNA sequencing.

Introduction

With the rapid urbanization and increasing population for a country like India, it is very important that the agricultural land should be protected and should remain free of every type of pollutant. Microbiology approaches is the best way for managing the environment[1]. Large – scale production, transports, use and disposal of petroleum have made it a leading contaminant in the environment[2]. Oil sludge and crude oil contamination is a major environmental concern because many of the constituent hydrocarbons are found to be toxic. In recent years the petroleum exploitation and production activities have increased which results in increased discharge of petroleum hydrocarbon into our environment [3]. The application of microbial isolates for degradation of crude oil involves the manipulation of environmental conditions to favour microbial growth and degradation to proceed at a higher rate[4,5]. Bioremediation of waste materials containing hydrocarbons and their derivatives, is depend on the ability of microorganisms to increase their population on these substrates and to degrade them to non-toxic products, such as H_2O and CO_2.[6,7]. Over the past two decades an increasing interest in the bioremediation of environmental pollutants through manipulation and application of degradative microorganisms has been shown[8,9,10]. I*n situ* and on-site both the kind of treatment involve use of microorganisms to degrade hazardous organic environmental contaminants avoid the economic and technical disadvantages[11]. The present study was focused on isolating crude oil degrading microbes from the soil samples collected from the oil field and their identification using 16S r-RNA sequencing method.

Material and Methods

Sample Collection: Soil samples of different depth were collected from the oil field ONGC, Ankleshwar, Gujarat. The samples were collected in sterilized sample containers. The soil samples were stored at 4^0C and immediately transferred to the laboratory for further analysis.

Isolation of Microbes: Bushnell Hass medium was used to isolate crude oil degrading bacteria. In the procedure, 10 grams of sample was added in 100mL of 0.9% N-saline. It was vortexed for 10 minutes and allowed to stand for a few minutes. Supernatant was collected in other sterilized tube to be used as inoculums. These inoculums were streaked on nutrient agar plates after serial dilution and the plates were incubated for 48 hours at 37^0C.

Enrichment of Microbes: For isolate the desired bacteria, enrichment culture technique was used. In the process, 1 mL of soil mixture was inoculated into 50 mL of Bushnell Haas broth containing 1% (v/v) crude oil as a sole carbon source. The flasks containing media and crude oil were incubated on a rotary shaker at 120 rpm at 37ºC for atleast 7 days. After incubation, a loopful of culture was streaked on Bushnell Haas agar containing 1% crude oil and incubated at 37°C for 24h-48h. Selective microbial colonies were picked and sub-cultured by streaking onto Bushnell Haas Plates to obtain pure culture.

Identification of Microbes: Identification of the crude oil degrading bacteria was done by 16s r-RNA sequencing method. Obtained sequences were submitted to NCBI database.

Results and Discussion

Among the various microbes obtained on the nutrient agar plates, selected colonies were able to grow on Bushnell Haas medium containing crude oil as a carbon source. This indicates that not all the microbes present in the sample are capable of

crude oil degradation. These microbes might be resistance of crude oil presence and hence survived in such environment. Among the various isolates obtained on the BH media, only four colonies were found to give growth within 24-48 hours as compare to others which has given visible growth after 96-120hrs. The growths of colonies of these microbes are depended on their capability of crude of degradation. Higher degradation librates more carbons in the media, which will be readily available to microbes for their growth and reproduction. Hence, based on the colony growth it was found that these four microbes are potential crude oil degraders.

Identification of these four isolates was done using 16s r-RNA sequencing method. They are identified as *Bacillus cereus, Pseudoxanthomonas mexicana, Halomonas daqingensis* and *Parapusillimonas granuli* respectively. The obtained sequences were submitted to NCBI with accession number KM192258, KM192259, KM192260 and KM192261 respectively. Some physiological properties of these microbes were also studied and the following observations were made, table–1.

Table-1
Physiological properties of isolated microbes

Organism	Gram Staining	Motility test	Spore formation
Pseudoxanthomonas Mexicana	Gram negative	Motile	Non-sporulating
Halomonas daqingensis	Gram negative	Non-motile	Non-sporulating
Bacillus cereus	Gram positive	Motile	Sporulating
Parapusillimonas granuli	Gram negative	Motile	Non-sporulating

Conclusion

It can be concluded from the above experiment that crude oil degrading potent bacteria could be easily isolated from crude oil contaminated sites and bioremediation could be make possible by using potential microbes obtained.

References

1. Prabakaran, V. Entitled as biodegradation and bioremediation of pesticides in an agro ecosystem, **(2004)**

2. Jegadevan S. and Mukberji, S. Successful in situ oil bioremediation programmes key parameters, *Indian J. Biotechnol.,* **3,** 495–501 **(2004)**

3. Kuberan T., Degradation and quantification of polyaromatic hydrocarbon by Pseudomonas sp., *Environ. Sci.,* **6, (2011)**

4. Das T.T., Gohel H.R., Panchal M.R., Ghosh S.K. and Braganza V.J. Determination of Crude Oil Degradation Efficiency of Glass Biofilm of Isolated Bacterium and Fungus, *Int. Res. J. Biol. Sci.,* **3,** 67–69 **(2014)**

5. Vidali M., Bioremediation : An overview, *Pure Appl. Chem.,* **73,** 1163–1172 **(2001)**

6. Toledo F.L., Calvo C., Rodelas B. and Lopez J.G., Selection and identification of bacteria isolated from waste crude oil with polycyclic aromatic hydrocarbons removal capacities, *Syst. Appl. Microbiol.,* **29,** 244–252 **(2006)**

7. Guru G., Gohel H., Ghosh S. and Braganza V., Isolation and Enrichment of Microbes for Degradation of Crude Oil., *Int. J. Eng. Sci. Innov. Technol.,* **2,** 144–147 **(2013)**

8. Vincent A.O., Felix E., Weltime M.O., Ize-iyamu O.K. and Daniel E.E., Microbial Degradation and its Kinetics on Crude Oil Polluted Soil, *Res. J. Chem. Sci.,* **1,** 8–14 **(2011)**

9. Nikhil T., Deepa V., Rohan G. and Satish B., Isolation, Characterization and Identification of Diesel Engine Oil Degrading Bacteria from Garage Soil and Comparison of their Bioremediation Potential, *Int. Res. J. Environ. Sci.,* **2,** 48–52 **(2013)**

10. Atlas R. and Bartha R., Biodegradation of oil on water surface : Polluting petroleum hydrocarbons, **(1984)**

11. Ahlert R.C. and Kosson D.S., In situ and on-site biodegradation of industrial land Wll leachate, **(1983)**