International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Special Issue 1- ICMTBT | 2018 | 80-83
International Conference on Microbial Technology for Better Tomorrow ICMTBT
Held at D.Y. Patil College of Arts, Commerce and Science, Pimpri, Pune, 17-19 February
| Conference Proceedings | Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

ISOLATION, IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF HYDROCARBON DEGRADING MICROORGANISM FROM CONTAMINATED SITES OF KOPARGAON, MAHARASHTRA

Kolkar M.V* and Kharat A.R

Department of Microbiology, Dr.D.Y.Patil Arts, Commerce and Science Women's College, Pimpri, Pune 411018 (Savitribai Phule Pune University), Maharashtra.

***Corresponding Author Email: madhurimakolkar20@gmail.com**

ABSTRACT

Environmental pollution caused by petroleum is at great concern because petroleum hydrocarbons are toxic to all forms of life. Oil spills have become a global problem particularly in industrialized and developing countries. Natural crude oil seepage and human activities are main cause of water and soil pollution. Oil contaminated soils were collected from the petrol pump of Kopargaon. These samples were screened for bacterial oil degradation using 0.5% petrol in Bushnel-Hass Mineral Salt Medium. It was concluded that out of the 7 isolates, three isolates MH -I, MH-II and MH-III were comparatively better and potent hydrocarbon degraders. Rate of biodegradation depends greatly on the composition and concentration of the oil or hydrocarbons. Temperature, oxygen and nutrient concentrations affect rate of biodegradation. Biochemical screening of MH -I, MH-II and MH-III were done. Simultaneously, antibiotic efficacy was also tested against ampicillin; catalase test, starch hydrolysis, sugar fermentation and urease test analysis were performed.

KEY WORDS

Hydrocarbon degradation, antibiotic susceptibility, urease production.

1. INTRODUCTION:

Hydrocarbons are the world's most widely used primary energy and fuel resources. The oil polluting compounds are light hydrocarbons (oil, petroleum, diesel), heavy hydrocarbons (heavy oil, crude oil, lubricants) and other more complex molecules. Cleaning up of petroleum hydrocarbon in the surface environment is a real-world problem.

Hydrocarbons are considered to be of biological origin, they are short and long chain hydrocarbons (alkanes: C_{10} – C_{20}; C_{20} – C_{40}) appear to be exclusively the origin of biological processes. These hydrocarbon contaminations are hazardous to the health of plants. Hydrocarbons are also carcinogenic, mutagenic and potent immuno-toxicants, causea serious problem to human and animal health.

Biodegradation of hydrocarbons by natural populations of microorganisms allows for the conversion of hazardous substances into less or non-toxic forms and represents one of the primary mechanisms by which petroleum and diesel products are removed from the environment inexpensively (Atlas, 1981; Floodgate, 1984; Leahy and Colwell, 1990; Lidderdale, 1993).

Microbial degradation process helps the removal of spilled oil from the environment after critical elimination of large amount of oil by various physical & chemical methods. This is possible because micro-organism have enzyme system they degrade and utilize petrol oil as a source of carbon and energy.

The present work has been focused on this approach, aiming to isolate novel bacterial strains capable of petroleum hydrocarbon degradation *in situ* conditions.

In this study, we report isolates capable of degrading a wide spectrum of hydrocarbons efficiently as well as we study biochemical characterization test of isolates such as starch hydrolysis, catalase, urease production antibiotic susceptibility and sugar fermentation capability. We isolate the most potent hydrocarbon degrading strains, which can be used for any bio augmentation studies during bioremediation.

2.MATERIAL AND METHODS

2.1 Collection of Sample

Soil samples were collected from contaminated sites of Kopargaon petrol pump, Ahemadnagar district, Maharashtra using a sterile spatula at a tillage depth of 1-2 cm, randomly from various points. The soil samples were collected into sterilized glass bottles, that bottles were properly sealed, labelled and wrapped to prevent any further light reactions. Temperature of collected soils ranged from 35-36°C. These samples were analysed for the presence of hydrocarbon degrading bacteria. The samples were preserved in the refrigerator until further use.

2.2. Culture Media

For enrichment of cultures and purification of isolates, Bushnel-Hass Mineral Salt Medium ('Practical Microbiology', R. C. Dubey and D. K. Maheshwari, 5thedition) was used by adding 0.5% petrol.

2.3. Isolation of hydrocarbon degrading microbes:

One gram of soil was taken from each sample and was added into 10 ml of distilled water. Dissolved soil sample was serially diluted to 10^{-2} and 10^{-3}. One ml soil suspension was taken and inoculated on the surface of Bushnel-Hass Mineral Salt Medium plate containing 100µl crude oil/petrol /100ml. Plates were incubated at ambient temperature for 24-48 hours. After incubation colonies developed were selected and sub cultured on the agar slants. The agar slants were preserved in the refrigerator after development of growth.

2.4. Secondary screening of oil/ petrol degrading isolates

All isolates obtained on Bushnel-Hass Mineral Salt Medium were further tested on the same medium by increasing the concentration of petrol. Only few organisms were capable to show the growth on the medium with 1% petroleum. Selected hydrocarbon degrading isolates were further identified by performing various biochemical tests.

2.5. Identification

Three best isolates designated as MH-I, MH-II and MH-III was identified by following techniques.

1. Gram staining. (Table No. 1)

3. Biochemical characterization of selected microorganisms: -

Biochemical tests, urease production, starch hydrolysis, carbohydrate fermentation (lactose), catalase test were performed with three selected isolates MH-I, MH-II and MH-III Medium ('Practical Microbiology', R. C. Dubey and D. K. Maheshwari, 5thedition).

3.1. Antibiotic Susceptibility test:

Ampicillin antibiotic was used for testing susceptibility of three isolates degrading hydrocarbon antibiotic susceptibility test was performed by agar well method ('Practical Microbiology', R. C. Dubey and D. K. Maheshwari, 5thedition) growth or no growth was recorded.

4. RESULT AND DISCUSSION:

Totally seven isolates utilizing hydrocarbon were obtained. Among these three isolates were detected as an efficient in secondary screening all these three isolates were tested for colony characters, morphological characters, grams nature, motility, biochemical characters and antibiotic sensitivity the results are presented in Table 4.1

4.1. Table 1: Morphological characteristics

Isolates	Gram Staining and Morphology
MH-I	Gram positive, cocci
MH-II	Gram positive rods
MH-III	Gram positive, cocci

4.1.2. Colony appearance on Bushnel-Hass Mineral Salt Medium

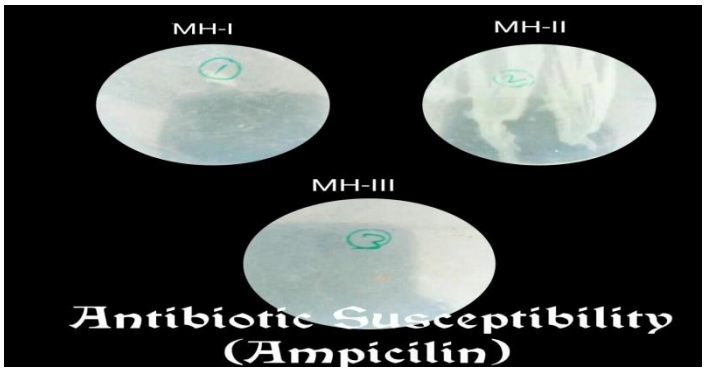
Fig -1 Three different isolates

4.2. Table 2: Biochemical testing

Biochemical Tests	MH-I	MH-II	MH-III
Starch hydrolysis	+	-	+
Catalase.	+	+	+
Urease production.	+	-	-
Antibiotic susceptibility	Susceptible	Resistant	Susceptible

4.2.3. Starch hydrolysis

4.2.4. Antibiotic susceptibility



DISCUSSION

The contaminated soil samples were enriched with the hydrocarbon degrading microorganisms and as we isolated totally seven isolates from the oil contaminated soil. Three efficient isolates out of the seven were selected for further studies.

The observation showed that all three isolates were gram positive. Biochemical tests like catalase test, starch hydrolysis, urease production and carbohydrate fermentation of MH-I, MH-II and MH-III were performed all isolates were showing catalase positive. Only MH-I was able to produce urease.

All three isolates possessed the best growth with oil/petrol as a sole source of carbon (1%).

We examined the antibiotic sensitivity against ampicillin. MH-III was found to be susceptible to antibiotic.

5.CONCLUSION

Environment pollution caused by released of a wide range of compound because of industrial progress. To prevent development of hazardous waste the process of bioremediation has been followed. Our present study follows the isolation of hydrocarbon degrading microorganisms from the various contaminated soil with petrol and diesel oil. Sample was collected from contaminated site of Kopargaon, Maharashtra.

Biochemical tests were performed with isolates. The isolates were screened for their oil degrading capacity. Antibiotic susceptibility was done with isolates.

Diversification of samples as well as media and hydrocarbons led to a potential large variety of isolates with biological activities, which may be different.

To explain these results, we can postulate. The nitrogen sources are responsible for providing nitrogen for the synthesis of cells' components. Since, in this media, we are not supplying amino acids, the addition of amino acids in the media may increase the growth rate, depending on the nitrogen source.

REFERENCES

1. Kvenvolden KA, Cooper CK (2003). Natural seepage of crude oil into the marine environment. *Geo-Marine Letters*. 23(3-4):140–146
2. Nalinee Kumari, Abhishek Vashishtha, Pooja Saini and Ekta Menghan isolation, Identification and Characterization of Oil Degrading Bacteria Isolated from the Contaminated Sites of Barmer, Rajasthan. *International Journal of Biotechnology and Bioengineering Research*. 4, (429-436)
3. Anupama Mittal & Padma Singh Isolation of hydrocarbon degrading bacteria from spills contaminated with crude oil spills. *Indian Journal of Experimental Biology* Vol. 47, September 2009, pp. 760-765
4. Zulfa AlDisi Samir Jaoua Dhabia Al-Thani Saeed AlMeer Nabil Zouari. Isolation, Screening and Activity of Hydrocarbon-Degrading Bacteria from Harsh Soils. Proceedings of the World Congress on Civil, Structural, and Environmental Engineering (CSEE'16).
5. April TM, Foght JM, Currah RS. (2000) Hydrocarbon degrading filamentous fungi isolated from flare pit soils in northern and western Canada. Canadian Journal of Microbiology.46 (1):38–49
6. Panda S. K, Kar R. N, Panda C. R, Isolation and identification of petroleum hydrocarbon degrading microorganisms from oil contaminated environment. International Journal Of Environmental Sciences Volume 3, No 5, 2013
7. Antai, S.P. and Mgbomo, E., (1993), Pattern of degradation of bonny light crude oil by Bacillus spp. and Pseudomonas spp. isolated from oil spilled site, W.A.J. boil. Appl. Chem. 38, pp 16-20.
8. Ganesh, A. and Lin, J., (2009), Diesel degradation and biosurfactant production by Gram-positive isolates. African Journal of Biotechnology, 8 (21), pp 5847-5854.
9. N. Das, and P. Chandran, Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview Biotechnology Research International, pp. 1-13, 2011.
10. R. Brewer, J. Nagashima, M. Kelley, M.Heskett, and M. Rig Risk-Based Evaluation of Total Petroleum Hydrocarbons in Vapor Intrusion Studies International Journal of Environmental Research and Public Health, vol. 10, no. 6, pp. 2441-2467, 2013.
11. R. Iturbe, and J. López, Bioremediation for a Soil Contaminated with Hydrocarbons Journal of Petroleum & Environmental Biotechnology vol. 6, no. 2, pp. 1-6, 2015.

***Corresponding Author:**
Kolkar M.V*
Email: madhurimakolkar20@gmail.com