SCREENING OF MICROORGANISMS FOR THE BIODEGRADATION OF HYDROCARBONS

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ABSTRACT

The present investigation is on checking the presence of biodegrading organisms on the sites of oil spilling with abundance of hydrocarbons. In order to proceed in our investigation, we need to isolate microorganisms from the sites of abundant hydrocarbon percentage and then to identify the organism isolated and culture them. The main goal of our investigation is to know which microorganisms having the highest and lowest capabilities to degrade the hydrocarbons effectively. Later on we will proceed by isolating plasmid from a high degrading efficient microorganism and transfer to the competent cells of lower efficient microorganism and test its capabilities to degrade the hydrocarbons.

INTRODUCTION

Biodegradation is a chemical breakdown of material into simpler compounds by microorganisms, where the microorganisms use organic material as carbon and energy source. The terms Degradation refers to decay and Bio refers that the degradation is carried out by various living organisms which are present in environment such as bacteria, fungi, and yeast, which decay the organic matter. Thus a biodegradable product is one which will decay naturally and organically (1).

Hydrocarbons, composed entirely of carbon and hydrogen, are the most important family of organic compounds. They are the organic compounds of simplest composition and may be considered the parent substances from which all other organic compounds are derived (2). The hydrocarbons are conveniently classified into two major groups: open-chain and cyclic. In open-chain compounds containing more than one carbon atom, the carbon atoms are attached to each

other to form an open chain; the chain may carry one or more side branches. In cyclic compounds the carbon atoms form one or more closed rings. The two major groups are subdivided according to their chemical behavior into saturated and unsaturated compounds (3).

Oil spill is a release of a liquid petroleum hydrocarbon into the environment due to human activity, and is a form of pollution. The term often refers to marine oil spills, where oil is released into the ocean or Oil also enters the marine environment from natural oil seeps. Most human-made oil pollution comes from land-based activity, but public attention and regulation has tended to focus most sharply on seagoing oil tankers (4).

Petroleum hydrocarbons are degraded primarily by bacteria and fungi. Biodegradation of petroleum require a mixture of microorganisms in the presence or absence of air with large quantities of compounds that are required for the growth of microorganisms. Adaptation by prior exposure of microbial communities to hydrocarbons increases hydrocarbon degradation rates (5).

Applications for genetically engineered microorganisms (GEM) in bioremediation have received a great deal of attention to improve the degradation of hazardous wastes under laboratory conditions. There are reports on the degradation of environmental pollutants by different bacteria. . The genetically engineered bacteria showed higher degrading capacity. However, ecological and environmental concerns and regulatory constraints are major obstacles for testing GEM in the field (6). These problems must be solved before GEM can provide an effective clean-up process at lower cost.

METHODOLOGY

Isolation of micro-organisms from soil sample: Collect soil sample from a highly concentrated hydrocarbon area (petrol pumps or vehicle service centers). Weigh 1gm of the soil sample and add in 10ml 0.89% saline water and name it as stock. Take 7 test tubes and add 10ml saline water in 1^{st} tube and fill the rest with 9ml of saline water. Take 1ml stock solution and add into the first tube and mix thoroughly this makes the dilution rate of 10^{-1}. Now keep transferring 1ml from previous 10ml test tube into the next test tube till dilution rate of 10^{-7} is achieved at 7^{th} tube. Prepare 40ml of nutrient media in a media bottle and wrap it. Take two clean Petri plates and wrap

them. Keep the media and plates for autoclave till 15psi. After autoclaving pour the media into the Petri plates and allow it to solidify. After solidifying spread 1ml each of 10^{-6} and 10^{-7} diluted sample using a spreader into the respective plates and label them. Incubate at 37^{o}c overnight. After incubation, colony formation was observed in the Petri plates.

An endospore is a dormant, tough, and non-reproductive structure produced by a small number of bacteria from the Firmicute phylum. Endospores are commonly found in soil and water, where they may survive for long periods of time. Bacteria in genera such as Bacillus and Clostridium produce quite a resistant structure capable of surviving for long periods in an unfavorable environment and then giving rise to a new bacterial cell. This structure is called an endospore since it develops within the bacterial cell. The location and size of endospores vary with the species; thus, they are often of value in identifying bacteria. Endospores are spherical to elliptical in shape and may be either smaller or larger than the parent bacterial cell. Endospore position within the cell is characteristic and may be central, subterminal, orterminal. Endospores do not stain easily but, once stained, they strongly resist de-colorization. This property is the basis of the Schaeffer-Fulton or Wirtz-Conklin method of staining endospores. The endospores are stained with malachite green. Heat is used to provide stain penetration. The rest of the cell is then decolorized and counterstained a light red with safranin. A stained preparation of Bacillus subtilis showing endospores as green and the vegetative cell as red

Screening of bacteria for biodegradation of hydrocarbon: Prepare RAMSAY media with glucose and another RAMSAY media without glucose in two separate media bottles. Wash the Petri-plates and air dry them. Wrap the bottles and Petri-plates and autoclave it at 15psi for 1hour. Clean the laminar air-flow with alcohol and switch on the U.V light for 5minutes. After autoclaving pour the RAMSAY glucose media into the Petri-plates which act as control in the laminar air-flow. Also add RAMSAY without glucose into the test Petri-plates and add the desired hydrocarbon (benzene, petrol, diesel, toluene, pyridine and hexane) and allow the media to solidify.

After the media is solidified streak the microorganism Bacillus sutilis, Bacillus lechinformis, vibrio species, Pseudomonas auroenosa, Pseudomonas flouro and Corny bacteria) from the respective pure cultures on to the Petri-plates. Incubate at room temperature for 2-3 days till

maximum growth is observed. Compare the control Petri-plates with the tests and observe the growth and note which bacteria is degrading which hydrocarbon easily.

RESULTS

Different colonies with different morphological characters have been formed. Microorganisms play an important role in the degradation of hydrocarbons. In order to observe the ability of microorganisms to degrade the hydrocarbons bacteria and pseudomonas species were isolated from the soil sample. These were identified on the basis of their morpho- cultural and biochemical characteristics. All isolated strains of bacteria and pseudomonas were screened for the ability of utilizing hydrocarbons including Benzene, Diesel, Petrol, Camphor, Toluene, Hexane, and Pyridine. Zone of degradation or zone of hydrolysis is observed surrounding the bacterial growth, indicates the utilization of carbon source obtained from petroleum products (Table 1).

The organisms *Pseudomonas fluorescence and Pseudomonas aeruginosa* are having the highest potential to degrade the hydrocarbon as compare to the other organisms. The Pseudomonas species are having the highest potential to degrade hydrocarbons when compared to the bacteria species and hence, the plasmid DNA from Pseudomonas species are transformed to the bacterial species to increase the potential of the bacterial species to degrade hydrocarbons under favorable conditions.

CONCLUSION

The organisms *pesudomonas fluorescence, pseudomonas aeruginosa* are having the hghest potential to degrade the hydrocarbons such as petrole, diesel, pyridine, etc, as compare to other to other organisms and bacillus have lowest potential to degrade the hydrocarbons. The degradation capacity of low degrading organisms can be increased by inserting the plasid of high degrading efficient micro-organism. Mixed culture strains of different species that can degrade petroleum and its products efficiently can be considered for efficient biodegradation of hydrocarbons. This is for the reason that not all microorganisms can degrade all the typrs of hydrocarbons. Biodegradation of hydrocarbons can be made faster and more efficient by engineered mutation of bacteria that can degrade the hydrocarbons.

Table 1: Sscreening of bacteria for degradation of hydrocabons

Micro organism	Hydrocarbon	Growth After 24 hours	3rd day
Bacillus subtilis *Bacillus licheniformis* *Vibrio species*	Diesel	+ ++ ++	++ ++ ++
Bacillus subtilis *Bacillus licheniformis* *Vibrio species*	Benzene	+ ++ ++	++ +++ ++
Bacillus subtilis *Bacillus licheniformis* *Vibrio species*	Petrol	+++ +++ +++	++ ++ ++
Bacillus subtilis *Bacillus licheniformis* *Vibrio species*	Camphor	+ ++ +	++ +++ ++
Bacillus subtilis *Bacillus licheniformis* *Vibrio species*	Toluene	+ ++ +	+ ++ ++
Bacillus subtilis *Bacillus licheniformis* *Vibrio species*	Pyridine	++ ++ ++	+++ +++ +++
Bacillus subtilis *Bacillus licheniformis* *Vibrio species*	Hexane	++ ++ ++	++ +++ ++
Pesudomonas fluorescence *Pseudomaeruginosa* *corny bacterium*	Diesel	+ + + + + -	+ + + + + + +
Pesudomonas fluorescence *Pseudomonas aeruginosa* *corny bacterium*	Benzene	+ + + + + + +	+ + + + + + + + +
Pesudomonas fluorescence *pseudomonas aeruginosa* *corny bacterium*	Petrol	+ + + + + + + + +	+ + + + + + + + +
Pesudomonas fluorescence *pseudomonas aeruginosa* *corny bacterium*	Toluene	+ + + + + + + + +	+ + + + + + + + +
Pesudomonas fluorescence *pseudomonas aeruginosa* *corny bacterium*	Pyridine	+ + + + + + + + +	+ + + + + + + + +

REFERENCES

1. Ajay, D. 2008. Studies on blister blight disease (Exobasidium vexans Massee) Infecting Tea. Ph.D thesis submitted to Bharathiar University, Coimbatore, Tamilnadu, India. Pp: 11.
2. Asamizu, E., Nakamura, Y., Sato, S. and Tabata, S. 2004. Characteristics of Lotus japonicus gene repertoire deduced from large-scale expressed sequence tag (EST) analysis. *Plant Molecular Biology*. 54. Pp: 405–414.
3. Baby, U.I. and Sanjay, R. 2004. Economic threshold level for grey blight disease. *Newsletter, UPASI Tea Research Institute*. 14(2). Pp: 4
4. Al-Taweel, K., Fernando, W.G. and Brule-Babel, A.L. 2011. Construction and Characterization of a cDNA Library from Wheat Infected with *Fusarium graminearum* Fg 2. *Int. J. Mol. Sci.* 12. Pp: 613-626
5. Ashikawa, I., Hayashi, N., Yamane, H., Kanamori, H., Wu, J. et al., 2008. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer pikm-specific rice blast resistance. *Genetics*. 180. Pp: 2267–2276.
6. Ano, A., Takayanagi, T., Uchibori, T., Okuda, T. and Yokotsuka, K. 2000. Characterization of a classIII chitinase from *Vitis vinifera* cv.Koshu. *J Biosci Bioeng.* 95. Pp: 645–647