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RESEARCH ARTICLE

PLASMID ENCODED GENES SPECIFYING DIESEL OIL DEGRADATION FROM *BACILLUS* SP AND *ENTEROBACTER HORMAECHEI*

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ABSTRACT

The capability of microorganisms to degrade an organic compound is the outcome ultimately of the hereditary composition of the organisms. The chemical reactions involved in metabolism are mediated by enzymes. The range of enzyme in the bacterium has a reflection of the specific genetic information in the cell. The genetic information in bacteria, as in all organisms, is stored in the form of DNA. The information is physically present in bacterial cells in two forms the chromosome and plasmids. The bacterial chromosome is a on its own circular, extremely folded double strand of DNA. In accumulation to chromosomal DNA, a bigger quantity of bacteria also has extra chromosomal DNA in the form of plasmids. Many plasmids contain genes which code for the enzymes necessary for the derivative pathways important to bioremediation. Enzymes occupied in the degradation of toluene, naphthalene, salicylate, octane etc. , have been exposed to be plasmid encoded. This paper deals with the isolation and identification of diesel oil degrading plasmid from diesel oil degrading bacteria.

Key words: Plasmid, Bioremediation, Bacillus sp, *Enteropacter hormaechei.*

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INTRODUCTION

A large number of bacteria with PAH degrading capabilities have been reported as able to either completely assimilate a defined range of compounds or carry out their transformation to different extents (Wattiau, 2002). Catabolic pathways, which encode different aromatic hydrocarbon degradation routes, are frequently located on plasmids, although degradative genes can be located on either chromosome or plasmid (Evans *et al.,* 1965; Kiyohara *et al.,* 1976; Barnsley 1983; Menn *et al.,* 1993). The ability of microorganisms to degrade an organic compound is the result ultimately of the genetic makeup of the organisms. The chemical reactions involved in metabolism are mediated by enzymes. The range of enzyme in the bacterium has a reflection of the specific genetic information in the cell. Genetic information in bacteria, as in all organisms, is stored in the form of DNA. The information is physically present in bacterial cells in two forms the chromosome and plasmids. The bacterial chromosome is a single circular, highly folded double-strand of DNA.

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In addition to chromosomal DNA, a larger number of bacteria also have extra-chromosomal DNA in the form of plasmids (Zylstra and Gibson, 1991). Many plasmids contain genes which code for the enzymes necessary for the derivative pathways important to bioremediation. Enzymes involved in the degradation of toluene, naphthalene, salicylate, octane etc. , have been shown to be plasmid encoded (Barbly and Barbour, 1984; Nelson, 1990). Plasmids are also important in the development (transformation) of new organisms with enhanced degradative capability. Using molecular techniques, it is possible to slice pieces of DNA containing genes for specific degradative pathways into plasmids. These plasmids can then be introduced into a host organisms resulting in a recombinant or Genetically Engineered Microorganisms (GEM) with new degradative capabilities (Brook and Madigan, 1991; Brand *et al.,* 1992). These are used to bioremediate contaminated sites mainly as organisms for bioaugumentation (McClune *et al.,* 1989; Philips *et al.,* 1989; Focht, 1998). Before isolation of plasmid having susceptible degradation gene, plasmid curing suggested by Trevors, (1986), Hardy(1993), Fujii *et al.,* (1997) and Sheikh *et al.,* (2003), were performed to show whether the oil degrading gene is plasmid encoded or chromosomal encoded. Plasmid DNA was isolated by the alkali lysis method (Kado and Liu, 1981; Sambrook and Russell, 2001). The agarose gel was stained with ethidium bromide and bands visualized on an UV transilluminator (Lodge *et al.,* 2007).

Molecular sizes of plasmids were determined by comparison with a super coiled DNA size marker (Sigma D5292) relying on the computer program DNA size version 1 (Raghava, 1994).

MATERIALS AND METHODS

Preparation of Hydrocrbon Degrading Strains

The bacterial strains of Bacillus sp and Enterobacter hormaechei were used in this study. This two isolates were maintained in Luria Bertani (LB) agar slants. The exponential phase cultures were grown in LB broth at 30°C for the isolation of plasmid DNA.

Luria Bertani Broth /Agar g/l

Tryptone	-	10
Yeast Extract	-	7
Sodium Chloride	-	10
Agar	-	*15*
Distilled Water	-	1000 ml
pH	-	*7*

Plasmid Curing

Plasmid curing was done with ethidium bromide and Sodium Dodecyl Sulphate (SDS) (Bopp *et al.,* 1983; Trevors, 1986). Exponentially growing culture was placed on LB agar plates containing different concentrations of curing agents (100 to 1000µg/ml) were inoculated and incubated at 37°C for 24 hrs. Exponentially growing cells in minimal medium were inoculated into sterile LB medium containing minimal inhibitory concentration (MIC) of the curing agents Ethidium bromide (650µg/ml) and SDS (4mg/ml) was incubated at 37°C. Cultures without treating with the curing agents served as control.

Plasmid DNA Extraction

The diesel degrading ability was genomic or plasmid borne, plasmid isolation was done for few isolates as described by Sambrook *et al.* (2001). Plasmid content of the sample was checked by Kieser's mini preparation method. 5ml of log phase broth culture was grown in LB with the antibiotic (chloramphenicol), centrifuged and resuspended in Tris buffer (Tris 25mM and sucrose 0. 3M) and cells in the Tris buffer were transferred to an eppendorf tube. 100µl of lysozyme solution (20 mg/ml of lysozyme in Tris) was added and incubated at room temperature for 30 min with intermittent mixing at the end of incubation period. 250µl of NaOH/SDS solution (0. 3M and 2% respectively) were added and mixed thoroughly in a vortex mixer.

The cap of the tubes were opened and incubated at 70°C for 10 minutes and then cooled at room temperature. 80 ml of phenol: chloroform mixture (Phenol 5gm, chloroform 5ml, water 1ml and 8 hydroxyquinoline 5mg) was added and vortexed till the phases mixed completely and spin at 5000 rpm for 5min. The aliquots of aqueous phase was transferred to new eppendorf tubes and washed twice with 70% ethanol. RNA was removed with RNase before use. Aliquots of the aqueous phase were electrophoresised in an 0. 9% of agarose gel for visualizing the plasmid DNA.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in submerged gel apparatus with TAE buffer (Tris-HCL - 10mm and EDTA - 1mm, pH 8) at a constant voltage of 3 volts/cm. After electrophoresis the gel was stained with ethidium bromide (0. 5mg/ml) for 20min and visualized under UV Transilluminator.

Running buffer TAE

50%	:	Working concentration
Tris base	:	0.04M Tris acetate
EDTA	:	0.01M EDTA
Water	:	1000ml
pH	:	8.3

RESULTS

The result of plasmid isolation showed that two isolates of Bacillus sp and Enterobacter hormaechei possess a single plasmid. The plasmids as revealed (Figure: 1) were of the same size (lanes 3 and 4). The strains had a band of approximately about 5. 5 kbp. The results also showed that the plasmids were successfully cured hence the disappearance of the bands was shown in lanes 1 and 2.

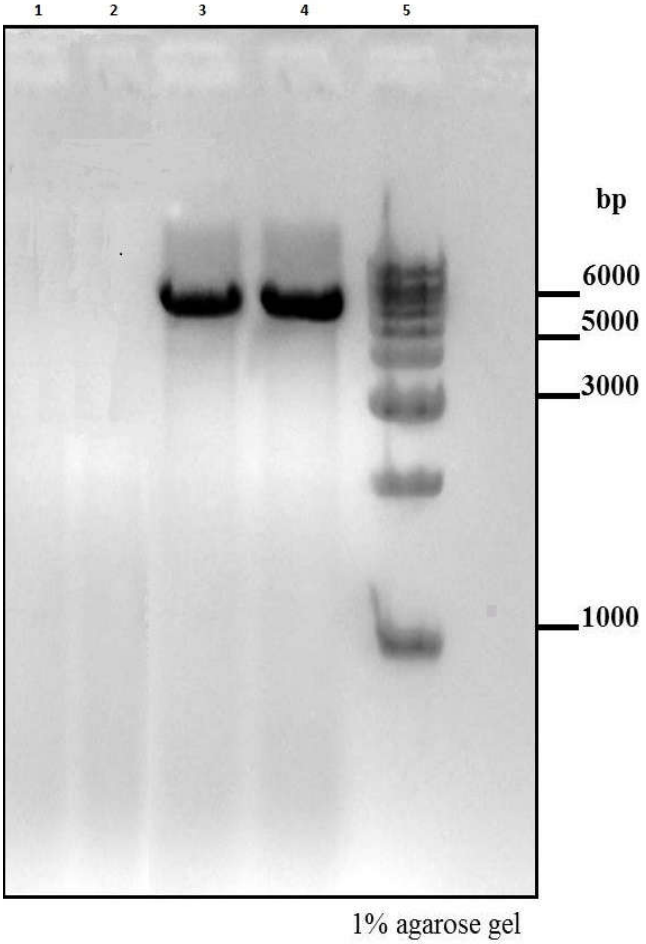

Figure 1. Plasmid profile of cured and non-cured isolates of Bacillus sp and Enterobacter hormaechei

Lane 1 - Cured isolate of Bacillus sp
Lane 2 - Cured isolate of Enterobacter hormaechei
Lane 3 - Non-cured isolate of Bacillus sp
Lane 4 - Non-cured isolate of Enterobacter hormaechei
Lane 5 - 1000bp DNA ladder

DISCUSSION

Oil spillage has become a global environmental problem. Natural bioremediation is the only eco-friendly solution to resist its devastating environmental and economic damage. The petroleum tolerant and degrading bacteria were isolated from different diesel oil contaminated soil samples. The diesel oil degradability and plasmid profile of diesel oil degrading bacteria, Bacillus sp and Enterobacter hormaechei isolated from diesel oil contaminated soil were studied. The ability of the isolates to degrade diesel oil was found to be plasmid mediated through curing experiment and agarose gel electrophoresis. Islam *et al.,* (2013) observed that the three petroleum degrading bacterial genera e.g., Micrococcus, Staphylococcus, Streptococcus were isolated from soil. The other three genera e.g., Bacillus, Klebsiella and Corynebacterium were isolated from water. A Staphylococcus sp isolated from soil was found to tolerate the highest concentrations of petroleum, therefore can be used potential to decontaminate oil spillage from water and soil. The role of plasmid in PAH degradation, the plasmid curing was conducted according to Ei-Mansi *et al.,* (2000) with some modifications. In this paper, the two bacterial isolates were subjected to plasmid curing. Two isolates were cultivated to exponential phase, and then 1 ml of culture was transferred to 4 ml of LB broth and 100 to 1000 µg/ml concentrations of SDS and Ethidium bromide. Bopp *et al.,* (1983), Trevors, (1986), Hardy, (1993) and Fujii *et al.,* (1997) also supported that the plasmid curing was conducted by using SDS and ethidium bromide as a curing agent. The bacterial isolates were grown in LB medium at 18^{o}C. The amount of Plasmid was extracted from overnight grown bacterial culture with the help of alkaline lysis method. In the same way, Ma *et al.* (2005) evaluated that 6 isolates (LCX 11, 12, 14 15, 16, 17) were cultivated in LB medium. The plasmids DNA were extracted from the selected isolates by the alkaline lysis method.

Conclusion

In this paper, the cured Bacillus sp and Enterobacter hormaechei grew successfully on diesel oil containing medium. The cured strains were lost their ability to degrade diesel oil. The study revealed that a relationship between diesel oil degradation and plasmid profile of hydrocarbon (diesel oil) degrading bacteria. It suggests that plasmid encoded gene could be involved in diesel oil biodegradation.

REFERENCES

Barnsley EA, 1983. 'Bacterial oxidation of naphtalene and phenanthrene'. *Journal of Bacteriology,* vol. 153, no. 2, pp. 1069 - 1071.

Bayly, RC and Barbour, MG, 1984. 'The degradation of aromatic compounds by the meta and gentisate pathways', vol. 13, pp. 253 – 294.

Bopp, LH, Chakrabarty, AM and Ehrlich, HL, 1983. 'Chromate resistance plasmid in Pseudomonas fluorescens', *Journal of Bacteriology,* vol. *155*, pp. 1105–1109.

Evans, WC, Fernley, HN, and Griffiths, E, 1965, 'Oxidative metabolism of phenanthrene and anthracene by soil pseudomonas', *Biochemical Journal,* vol. 95, no. 3, pp. 819 - 831.

Focht, DD, 1998. 'Performance of biodegradative microorganisms in soil; xenobiotic chemicals as unexploited metabolic niches', Environmental Biotechnology, Plenum Press, New York. pp. 15 - 30.

Fujii, Takeo and Maeda, 1997. 'Plasmid-encoded genes specifying aniline oxidation from Acinetobacter sp. Strain YAA', *Microbiology*, vol. *143, pp.* 93 – 99.

Hardy, KG, 1993. 'Plasmids, a practical approach', IRL Press, New York.

Islam, Ghosh, Magnet, Fatema, Akter, Khan, and Datta, 2013. 'Isolation and identification of petroleum degrading bacteria from oil contaminated soil and water and assessment of their potentially in bioremediation', *IOSR Journal of Environmental Science, Toxicology and Food technology,* vol. 5, no. 2, pp. 55 - 58.

Kado, CI, and Liu, ST, 1981. 'Rapid procedure for detection and isolation of large and small plasmids', *Journal of Bacteriology,* vol. 145, no. 3, pp. 1365- 1373.

Kiyohara, H, Nagao, K, and Nomi, R, 1976. 'Degradation of phenanthrene through o-phthalate by an Aeromonas sp', *Agricultural and Biological Chemistry,* vol. 40, no. 6, pp. 1075 - 1082.

Ma, Liu, and Shao, 2005. 'Isolation of phenanthrene-degrading bacteria and analysis of their degrading-enzyme gene', *Chinese Journal of Applied and Environmental Biology,* vol. 11, no. 2, pp. 218 – 221.

McClune, NC, Weightman, AJ, and Fry, JC, 1989. 'Survival of Pseudomonas putida UWCI containing cloned catabolic genes in a model activated – sludge unit', *Applied Environmental Microbiology, vol*. 55, pp. 2627-2634.

Menn, FB, Applegate, M, and Sayler, GS, 1993. 'NAH plasmid-mediated catabolism of anthracene and phenanthrene by naphthoic acids', *Applied Environmental Microbiology*, vol. 59, pp. 1938 - 1942.

Nelson, AH, 1990. 'The biodegradation of halogenated organic compounds', *Journal for Applied Bacteriology,* vol. 69, issue. 445 - 470.

Philips, SJ, Dalgarn, DS, and Young, SK. 1989. 'Recombinant DNA in wastewater. pBR 322 degradation kinetics', *Journal of the Water Pollution control Federation*, vol. 61, pp. 1588 - 1595.

Raghava, GP, 1994. 'Improved estimation of DNA fragment length from gel electrophoresis data using a graphical method', *Biotechniques*, vol. 17, no. 1, pp. 1000 - 1004.

Sambrook, J and Russell, D, 2001. 'Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press.

Sheikh, AR, Afsheen, A, Sadia, K and Abdu, W, 2003. 'Plasmid borne antibiotic resistance factors among indigenous Klebsiella', *Pakistan Journal of Biological Sciences,* vol. 35, no. 2, pp. 243 – 248.

Trevors, J. T, 1986. 'Plasmid curing in bacteria', FEMS *Microbiology Review,* vol. 32, pp. 149 - 157.

Wattiau, P, 2002. 'Microbial aspects in bioremediation of soils polluted by polyaromatic hydrocarbons', Focus on Biotechnology, vol. 3A, pp. 2 – 22.

Zylstra, GJ and Gibson, DT, 1991. 'Aromatic hydrocarbon degradation: A molecular approach', *Genetic Engineering,* vol. 13, pp. 183 – 203.

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