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ISOLATION OF BACTERIA OF *PSEUDOMONAS* AND *ENTEROBACTER* Spp. FROM SOIL AND STUDY OF THEIR ABILITY TO DEGRADE ORGANIC POLLUTANTS

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Abstract: Fifteen isolates of *Enterobacter* and *Pseudomonas* spp. were checked for their ability to degrade naphthalene, salicylate and benzoate. Seven of the isolated strains were able to degrade all three hydrocarbons, three could degrade two hydrocarbons while six of the strains degraded only one of the hydrocarbons. The hydrocarbons used in the study are usual pollutants from industrial and urban waste. The possibility of the use of these bacteria in degrading environmental pollutants is discussed.

Key words: *Pseudomonas*, *Enterobacter*, hydrocarbon degradation, hydrocarbon pollutants, aromatic hydrocarbons.

INTRODUCTION

Aromatic compounds are abundant in the biosphere as products of plant metabolism, wastes from industry, and as pollutants. Chlorinated aromatic compounds are usually toxic and are source of health hazards for animals and human beings due to their mutagenic and carcinogenic effects. A number of bacterial strains have been isolated from soil and waste-waters which play an important role in degrading organic compounds. These bacteria are capable of using aromatic compounds as sole source of their energy requirements and thus break down aromatic compounds. Many of these microorganisms degrade chlorinated benzoic acid, chlorinated phenols, chlorinated phenoxyacetic acid, and chlorinated benzene through a common pathway to ß-ketoadepate (Reincke and Knackmuss, 1988; Eaton and Chapman, 1992).

Study of the metabolic pathways involving breakdown of chlorinated hydrocarbons is usually undertaken with the objective that the elaboration of these pathways would lead to use of bacteria and the degradative enzymes in breaking down more and more toxic chlorinated hydrocarbons which are constantly appearing in the environment due to agricultural industry or other industries and due to dumping of wastes. In this way much information has collected about the enzymology and molecular regulation of aerobic pathways of aromatic compound degradation (Dagley, 1986; Harayama and Neidle, 1992; Ornston *et al.*, 1990; van der Meer *et al.*, 1992; Harwood *et al.*, 1994). It is important that many of the catabolic enzymes involved in these pathways are similar and the gene clusters that encode them are organized in similar fashions (Daubaras and Chakrabarty, 1992).

The objective of the present study was to isolate bacterial strains from local soil samples capable of degrading organic contaminants, like naphthalene, salicylic acid and benzoate. It was also aimed at checking the possibility of exploiting locally isolated strains for their hydrocarbon degrading potential.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from petrol pumps (service stations), in the city of Lahore, Pakistan using sterile vials and brought to the laboratory. The isolates were identified by staining and biochemical techniques.

Hydrocarbon degradation

Isolated strains of *Pseudomonas* and *Enterobacter* spp. were tested for their degradative capabilities by growing the cells on M9 salt medium having benzoate, salicylic acid or naphthalene as the carbon source for the bacteria. Agar plates were used for checking the colony formation.

Media

For selection of bacteria for their ability to metabolize aromatic hydrocarbon M9 medium was used but glucose was substituted by the respective hydrocarbon. The medium was prepared by dissolving 0.6g Na_2HPO_4, 3g KH_2PO_4, and 1g NH_4Cl in distilled water so that the ultimate volume of the medium was one liter. The solution was autoclaved. One ml of 1M $MgSO_4.7H_2O$ and 10ml of 0.01M $CaCl_2$ were autoclaved separately and mixed. When the temperature lowered to about 60°C, benzoate and salicylate were added instead of glucose. The pH of all the solutions was adjusted to 7.0. Naphthalene was sprinkled on agar plates so that its vapors were available to the bacteria for their growth. Agar plates were incubated at 30°C and colonies were observed after 24 hours.

RESULTS AND DISCUSSION

Various strains isolated from petrol pumps and different hydrocarbon contaminated soils were collected and grown on LB plates. After the appearance of growth these strains were checked for their naphthalene, salicylate or benzoate degrading ability. The culture of the respective strains was spread on plates containing M9 medium in which glucose was substituted with one of the hydrocarbons. Fifteen strains were screened in this way. The growth and appearance of colonies showed the ability of the strain to metabolize hydrocarbon present in the medium. The degradative capability of various strains is given in Table I.

Pseudomonas and *Enterobacter* strains isolated from soil samples exposed to petroleum spillage for a long duration harbored hydrocarbon degrading bacteria. Local strains are important in various kinds of operations which are especially beneficial for the local environment, like detoxification of chlorinated aromatic compounds present in the ecosystem. As these bacteria have been exposed to these chemicals for a long time hence they have developed metabolic pathways for degradation of the chemicals. This capability might have been acquired through the process of mutation and natural selection. *Pseudomonas* have some special genes related to catabolism of hydrocarbons.

which are not found in other bacteria. That has provided these bacteria the opportunity to survive in environment contaminated with hydrocarbons. Parallel to that, the scientists have got the opportunity to use these bacteria for the breakdown of certain toxic compounds.

All the fifteen strains of *Pseudomonas* and *Enterobacter* spp. were able to degrade and grow on benzoate, seven of the strains were capable of degrading all three hydrocarbons used in this study, three of the strains were able to catabolize two hydrocarbons and six were capable of degrading only one (Table I). The results show a widespread occurrence of hydrocarbon degradative pathways in many of the bacterial strains, particularly in those which are exposed to contaminated soils or waste-water. Naphthalene constitutes about 10% of the contaminants of our environment. The degradative capacity of a number of strains isolated in this study opens the opportunity for the environmentalists to exploit these bacteria for environmental cleanup. Chlorinated aromatic hydrocarbons are highly toxic, mutagenic and carcinogenic. Microbiological processing of these compounds would lead to cleaner environment with less secondary pollution resultant of any chemical operation.

Table I. Growth of various isolated strains of *Pseudomonas* and *Enterobacter* spp. on different hydrocarbon sources

Strain	Growth on benzoate	Growth on salicylate	Growth on naphthalene
ATCC 50208	+++	+++	+
Pseudomonas			
PS69	+++	+++	++
PS75	+++	+++	++
P2520	+++	+++	++
P2556	+++	+++	++
P2587	+++	+++	++
P2588	+++	+++	++
J49	+	+	++
Enterobacter			
A3	++	-	ND
A5	+	-	ND
B12	++	-	ND
B13	++	-	ND
E28	++	-	ND
E38	+	-	ND
D20	++	-	ND
D36	+	-	ND

Abbreviation / signs used: -, no growth; +, growth after 3 days; ++, growth after 2 days, +++, growth after 24 hours; ND, not determined.

REFERENCES

DAGLEY, S., 1986. Biochemistry of aromatic hydrocarbon degradation in pseudomonads, *In*: The bacteria, vol. 10. The biology of *Pseudomonas* (ed. J.R. Sokatch), pp. 527-556. Academic Press, Inc., Orlando.

DAUBARAS, D.L. AND CHAKRABARTY, A.M., 1992. The environment, microbes and bioremidiation: microbial activities modulated by the environment. *Biodegradation*, **3**: 125-135.

EATON, R.W., AND CHAPMAN, P.J., 1992. Bacterial metabolism of naphthalene: construction and use of recombinant bacteria to study ring cleavage of 1,2-dihydroxynaphthalene and subsequent reactions. *J. Bacteriol.*, **174**: 7542-7554.

HARAYAMA, S., KOK, M. AND NEIDLE, E.L. 1992. Functional and evolutionary relationships among diverse oxygenases. *Annu. Rev. Microbiol.*, **46**: 565-602.

HARWOOD, C.S., NICHOLAS, N.N., KIM, M., DITTY, J.L. AND PARALES, R.E., 1994. Identification of the *pcaRKF* gene cluster from *Pseudomonas putida*; involvement in chemotaxis, Biodegradation, and transport of 4-hydroxybenzoate. *J. Bacteriol.* **176**: 6479-6488.

ORNSTON, L.N., HOUGHTON, J., NEIDLE, E.L. AND GREGG, L.A., 1990. Subtle selection and novel mutation during evolutionary divergence of the ß-ketoadepate pathway. In: *Pseudomonas*: biotransformations, pathogenesis, and evolving biotechnology, *In*: eds. S. Silver, A.M. Chakrabarty, B. Iglewski, and S. Kaplan, pp. 207-225. American Society for Microbiology, Washington, D.C.

REINCKE, W. AND KNACKMUSS, H.J., 1988. Microbial degradation of haloaromatics. *Annu. Rev. Microbiol.*, **42**: 263-287.

VAN DER MEER, J.R., deVOS, W.M., HARAYAMA, S. AND ZEHENDER A.J.B., 1992. Molecular mechanisms of genetic adaptation to xenobiotic compounds. *Microbiol. Rev.* **56**: 677-694.

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