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STUDY OF GENETIC RELATEDNESS AMONG *Arthrobacter* STRAINS BY PHLYLOGENETIC ANALYSES OF RAPD-PCR FINGERPRINT PATTERNS

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ABSTRACT:

The genus *Arthrobacter* is aerobic, high GC containing, Gram positive bacteria categorized into the class Actinobacteria, order Actinomycetales, and family Micrococcaceae. They exhibit nutritional dynamism, and are capable of using a wide range of xenobiotoc compounds as energy source.They are competent enough to biodegrade serious environmental pollutants and atrazine containing herbicides. Genetic diversity among *Arthrobacter sp*. was determined by analysing their RAPD fingerprint patterns using NTSYS pc software package and UPGMA cluster method. Considerable amount of genetic diversity was observed among the isolated strains. Furthermore, dendrogram demonstrated the existence of three distinctive clusters, first cluster consisted of strain B3 that separates from B7 at 85.5% similarity level. Closely related strains B11, B10, B15, B9, B8 fall within same similarity level. Similarly B12, B13, B14 also form same similarity group with 85.5% similarity. The study was helpful in characterizing *Arthrobacter sp*. which are potentially important candidates for application in bioremediation due to their remarkable resistance to toxic compounds.

Keywords: RAPD-PCR, *Arthrobacte*r, NTSYS, Phylogenetic, Jaccords's Coefficient, UPGMA

INTRODUCTION:

Arthrobacter is a ubiquitous soil dwelling eubacterium They are also found in extreme environments, such as deep subsurface soils, arctic sea, radioactive waste tanks and include salt, psychrotolerant species [5]. They display exceptional nutritional versatility and are proficient in using a wide range of compounds as carbon source and many of the species are potential bioremediation agents. They are competent enough to biodegrade serious environmental pollutants like endothal, nicotine, 2,4D-nitroglycerine, 4, chlorophenol [4], phenolic mixtures, atrazine containing herbicides [9] and hexavalent chromium [12] and toxin-Swainsonine. The genus *Arthrobacter* is characterized as aerobic, Gram positive with high GC content. Some species of *Arthrobacter* have been reported to degrade Swansonine which leads to locoism [13]. Preliminary studies indicate that *Arthrobacter aurescens* TC1 is significantly resistant to ionizing radiations in the laboratory (M. Daly, unpublished data). Information regarding genetic diversity of indigenous *Arthrobacter*

strains can provide basis for selecting isolates for bioremediation. PCR based DNA fingerprinting methods such as RAPD and rep-PCR [7] provide a practical and effective means to examine genotypic diversity among *Arthrobacter* species. Study on genetic diversity of *Arthrobacter* strains isolated from terrestrial deep-subsurface sediments on the basis of 16S rRNA and *rec* A gene sequences showed that the deep subsurface isolates from the same stratum are largely monophyletic [6]. The present study was initiated to identify and characterize a consortium of *Arthrobacter* strains isolated from forest soil and to asses their diversity by RAPD-PCR approach.

[II] MATERIALS AND METHODS

2.1 Sampling site

Soil samples were collected from fifteen sites in forest area of Raisen district, Madhya Pradesh which lies in central part of India. Eight samples contained surface soil (SS) and seven samples contained soil at 20 cm depth (DS). Surface litter was scraped away and soil was passed through 2 mm sieve to have homogenous particles. Samples were stored in presterilized HDPE bags at 4^0C.

2.2 Bacterial strains and their identification

For isolation of strains serial dilution of homogenised samples was made in phosphate buffer. Briefly, 10 g soil samples were suspended in PBS and well stirred, 10 ml soil derived supernatant was firstly co cultured with 90 ml LB medium for 24 hours and the resulting bacterial suspension was mixed with atrazine at ratio of 1:9 (v/v). The mixture was incubated at 30^0C and 160 rpm for four days. After sub-culturing the bacterial suspension was serially diluted and spread over agar plates with 200 mg/l atrazine. Colonies were further confirmed by morphological and biochemical tests. After confirmation as *Arthrobacter* strains the colonies were maintained and used for further study.

2.3 DNA extraction

Total genomic DNAs from all strains were isolated using standard phenol-chloroform-isoamyl extraction and ethanol precipitation in the presence of sodium acetate (0.3 mol/L). The pellets were washed with 70% ethanol, dried and redissolved in 150 μL of TE buffer. The concentration and purity of DNA were estimated spectrophotometrically at 260 and 280 nm, respectively.

2.4 RAPD genomic fingerprinting

Eight arbitrarily chosen primers (P1 to P6 and OPA 9, OPB1) used for RAPD fingerprinting were 10 nucleotides in length and had a GC content of 70%. The reaction mixtures were incubated for 5 min. at 95^0C for initial denaturation and then amplified for 35 cycles consisting of 30s at 94^0C, 30s at 360C and 1 min. at 72^0C followed by 7 min. incubation at 72^0C. The amplification products were separated by 2% agarose gel electrophoresis and visualized under UV transilluminator.

2.5 Data analysis

RAPD-PCR fingerprint patterns were coded in the binary form, and analysed using NTSYS-pc package [11]. A simple matching coefficient was calculated to construct a similarity matrix and UPGMA algorithm was used to perform hierarchical cluster analysis and to construct a dendrogram.

[III] RESULTS:

3.1 Identification of *Arthrobacter* strains

With the aid of selective media fifteen *Arthrobacter* strains (B1 to B15) were isolated from surface and deep soil sediments of forest area of Raisen district. Strains were subjected to various biochemical and physiological tests for further confirmation.

3.2 RAPD-PCR analysis

All Arthrobacter isolates were further genotypically characterized by RAPD fingerprinting. Total genomic DNAs were amplified with eight oligonucleotide primers. The amplification patterns revealed a high level of polymorphism. Fingerprinting resulted in multiple DNA products with 03 to 10 bands ranging from 500 bp to 900 bp. The primers produced up to eight distinct PCR products per strain. An example of RAPD fingerprint is shown in Fig. 1. On evaluating genetic profile using NTSYS software programme with Jaccord's coefficient and UPGMA cluster method, it showed three distinctive clusters

Fig. 2, confirming genetic variation. First cluster consisted of strain B3 that separates from B7 at 85.5% similarity level. Closely related strains B11, B10, B15, B9, B8 fall within same similarity level. Similarly B12, B13, B14 also form same similarity group with 85.5% similarity.

[IV] DISCUSSION:

Genetic structure of natural microbial populations depends on environmental factors, creating patterns of genetic variability and effecting evolutionary change. The physical and chemical conditions of the environment act directly on microbes and therefore play an important role in determining genetic structure of their populations. The collected bacterial isolates from forest bed (08 surface soil samples and 07 samples contained soil at 20 cm depth) were analyzed by random amplified polymorphic DNA PCR (RAPD). The DNA of all the isolates was purified by the method of [8] and phylogenetic analysis under standard conditions was performed.

The total isolated genomic DNA of *Arthrobacter aurescens* after purification from all the fifteen strains were amplified using primer OPA9 (5'-GGGTAACGCC-3') The amplification pattern of isolated genomic DNA of 15 strains was examined following gel electrophoresis with 1.6% agarose gel to have distinctive separation fragments of genomic DNA. The bands produced in each of the patterns by RAPD method were analyzed by Jaccord's Coefficient. The above data can be used to further characterize *Arthrobacter sp.* isolated from various environmental samples. Their abundance in soils of various types and in different geographical locations attribute to their numerical predominance, coupled with nutritional versatility of commonly occurring species [2,1], suggests that they may be important agents of mineralization in soil and possibly also in some other habitats. *Arthrobacter* strains are metabolically diverse, and are capable of catabolising a variety of xenobiotic compounds. Putative *Arthrobacter* species have also been reported to lyse yeast cells [3] and mycelium of *Fusarium roseum*, a carnation-root pathogen [10]. Due to their metabolic diversity, *Arthrobacter sp*. have been used in industrial applications and are currently being used in the bioremediation of contaminated ground water.

An understanding of the structure of deep-surface bacterial populations and how it is related to the structure of the environment would help direct deep-subsurface bioremediation efforts, as well as help direct the search within the deep subsurface for microbes that posses unique characteristics for biotechnology applications.

[V] CONCLUSION:

Soil is one of the most poorly understood research habitats on earth. The functioning of this dark layer on the surface of the earth is vital for the survival of biosphere in its present form Microbial diversity in soil ecosystems exceeds by far, that of eukaryotic organisms. The number, diversity and distribution of microorganisms reflect overall soil productivity. One gram of soil may harbor upto 10 billion microorganisms of possibly thousands of different species. As less than 1% of the microorganisms observed under the microscope are cultivated and characterized, soil ecosystems are, to a large extent remains uncharted. The present study is designed to investigate the involvement of microflora especially bacterial community. The present research will help in exploration, identification, classification and conservation of genetic diversity of microbes with reference to forest soil of Raisen district of Madhya Pradesh. The development of such data offers immense opportunities for increasing the productive potential of the existing forests products.

Arthrobacter strains are metabolically diverse, and are capable of catabolizing a variety of xenobiotic compounds, including glyphosate, methyl tert-butyl ether, 2, 4-dichlorophenoxyacetate (2, 4-D), nicotine, 4-nitrophenol, dimethylsilanediol, fluorine, phthalate, nitroglycerine and diverse s-triazine

compounds. Other roles that have been ascribed to some *Athrobacter sp*. are phytoharmone production and dinitrogen fixation. These molecular studies will help us understand the different varieties of species of microbes that are present locally and how distinct are particular microbial populations from one another. The recent technologies related to genetic diversity study will also provide the scope of widening the base of the utilization of biological resources. Therefore the conservation of genetic diversity with special to microbes will certainly be a valuable contribution.

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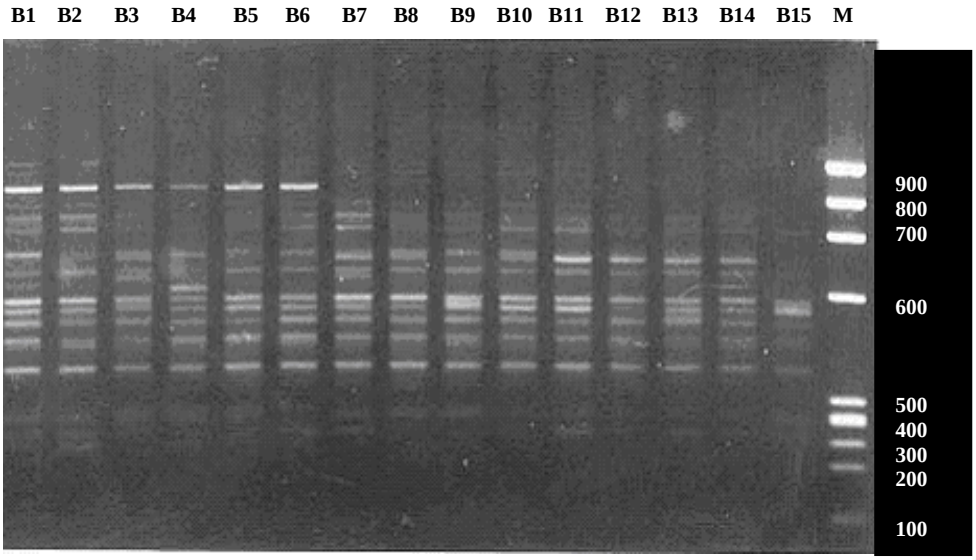

Fig: 1 RAPD PCR of A*rthrobacter aurescens* isolates from forest soil of Raisen district with primer OPA 9.

M – Marker
B1 to B8 – Surface soil isolate
B9 to B15 – Deep soil isolate

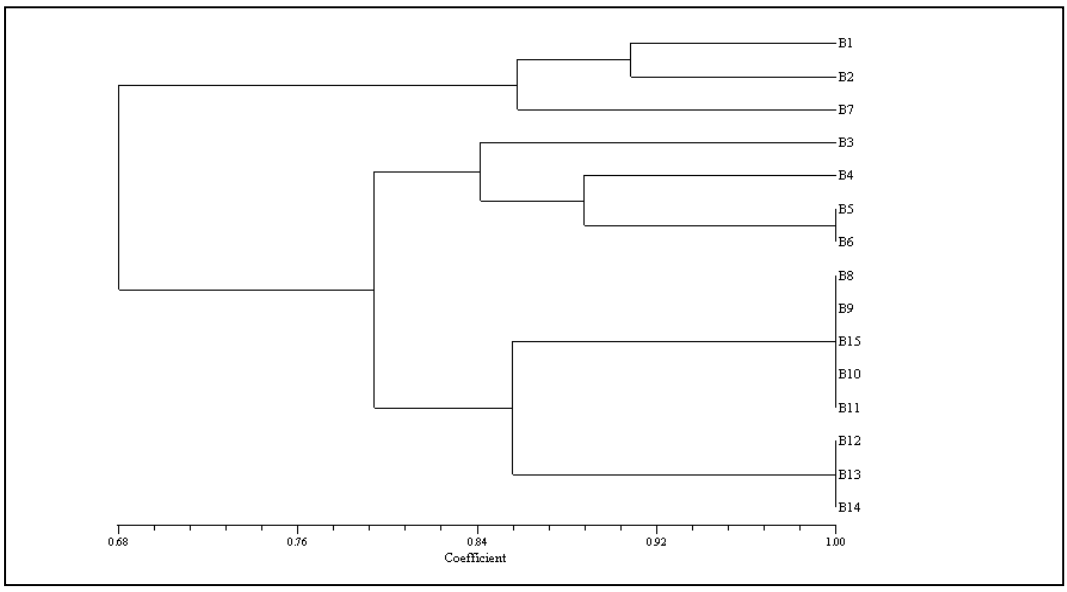

Fig: 2 Dendrogram showing genetic relatedness of 15 strains of *Arthrobacter aurescens* from forest soil of Raisen District determined by analysis of RAPD- PCR fingerprint patterns using Jaccord's similarity Coefficient and UPGMA cluster method**.**