RESEARCH PAPER

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Study the genetic variations among the *Azotobacter chroococcum* isolates using randomly amplified polymorphic DNA (RAPD) marker

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The geographical area of Karnataka is classified into ten agroclimatic zones. The first species of the genus *Azotobacter* named *Azotobacter chroococcum,* was isolated from the soil.The investigation was carried out to study the genetic variation of *Azotobacter chrococcum* occur in the soils of the ten different agroclimatic zones of Karnataka. These isolates were characterized by using RAPD markers. A total of 103 bands were scored out of which 87 bands were found to be polymorphic (84.97%). Statistical analysis of RAPD data enabled the classification of 10 *A. chroococcum* isolates in to three major groups. The RAPD banding pattern of these isolates could easily distinguish the isolates of different zones. In this the cluster analysis based on 103 RAPD bands revealed that the ten *A. chroococcum* isolates examined, clustered at a linkage distance of about 55 units on the dendrogram.

Key words : *Azotobacter chrococcum*, Waksman No. 77 N-free medium, RAPD, OPB-12, Hind3 marker,OPD-05.

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INTRODUCTION

The geographical area of Karnataka is classified into ten agroclimatic zones. Each zone has its own characteristic feature in relation to climatic condition, soil type, vegetation which has influence on the establishment of diversified flora and fauna. Soils form an excellent cultural media for the growth and establishment of many kinds of microorganisms. A gram of soil contains millions of microorganisms. The number and kinds of organisms present in soil depend on the nature of the soil, depth, season, state of cultivation, pH, organic matter content, temperature, moisture, aeration etc.

Azotobacter spp. are Gram negative, aerobic, free living nitrogen fixing bacteria that play an important role in improving plant growth and yield. The first species of the genus *Azotobacter* named *Azotobacter chroococcum,* was isolated from the soil. *Azotobacter* are widely distributed in non-acidic soils of India. *Azotobacter* have been used for studying nitrogen fixation and inoculation of plants due to their influence on growth and high level of nitrogen fixation. Several studies have revealed the beneficial effect of these bacteria in the improvement of crop growth and yield and Genetic diversity also estimated at molecular level. For genetic diversity estimation species characterization of DNA fingerprinting techniques are used for an extremely wide variety of applications. Molecular analysis of genomic DNA of the organism is useful for distinguishing the bacterial strains better at intra-species level. These techniques provide valuable information on the magnitude of genetic variability within and between organisms of different species. With the advent of molecular DNA techniques, several arbitrary primers based randomly amplified polymorphic DNA (RAPD) technique has been used for typing and identification of number of closely related species of bacteria and assessment of genetic relationships. Its results are usually consistent with those of DNA-DNA homology studies and can be used to estimate the genetic diversity.

RAPD uses the polymerase chain reaction to amplify DNA samples with short oligonucleotide primers that anneal randomly through out the genome. The result is a distinctive set of amplification products. Differences are visualized by staining the gel after electrophoresis of amplification products. Use of different oligonucleotide