Biogeography and Polyphasic Approach of Pseudomonas strains from Agriculture land of Madhya Pradesh

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Abstract

The present study related to microbial isolation of Pseudomonas species from different district of Madhya Pradesh helps to conclude bacterial species with similar biochemical property having approximately common genetic content exhibit molecular diversity to evaluate and compare the new Miniprimer-PCR to the two DNA-based typing techniques and the phenotypic characteristics for the purpose of assessing the diversity of microbial community isolated as collection of fluorescent pseudomonads. All the 50 strains of pseudomonas sp such as Pseudomonas aeruginosa, P.putida, P. fluroscence, and P. streuzi were shown to have genetic similarity with variation in arrangement of nucleotides in genome. Expression of species within the genus is regulated by reading frame of organism, therefore, with common concentration of DNA most of the species shows architectural motive once NTSYS prediction were made using UPGMA .Ornamentation of genome represent base substitution thus defining evolutionary distance along with constancy of character within species. All the Pseudomonas isolated looking into their biochemical profile can be used as PGPR and biocontrol agents.
Keywords: PCR , DNA,UPGMA Microbial diversity genetic similarity.

1. Introduction

One of the most important and best-studied bacterial taxa in soil is the genus Pseudomonas Noura, K M et al (2009). This genus includes several functional groups of environmental interest, such as plant growth promoters, (Patten, C L and B R Glick 2002) plant pathogens (Samson R et al 1998) and xenobiotic degraders (Clausen, G B 2002). Moreover, Pseudomonas species can also play important roles as biological control agents against soil-borne plant pathogens (Siddiqui, Z A 2006 and Nezarat, S and A Gholami, 2009). P. fluorescens is commonly found in soil and water, associated with food spoilage, in clinical specimens and also in association with plants and this species can be divided into five biovars (Soleimani, M J 2005). Microbial DNA fingerprinting has been reported extensively using PCR-based techniques such as Random Fragment Length Polymorphism (RFLP) and is now in common use. Among PCR-based molecular markers, RAPD (random amplified polymorphic DNA) and repetitive sequence-based PCR genomic fingerprinting have been found to be particularly efficient for bacterial analysis (Xu, R et al 2010). Traditional classifications based on phenotypic features do not always correlate with the molecular taxonomy (Woese C R 1987).

The 16S rDNA gene is a highly preserved region with small changes that can be characteristic of different species. Ribosomal genes are compared in most taxonomical studies of bacteria. Classical microbiological taxonomy traditionally used morphological and physiological differences among the species to discriminate between them. The biochemical tests could only discriminate at the species level, although physiological methods would not be able to distinguish the currently described species. At the genus level, several characteristics can contribute to the differentiation.

RFLP fingerprinting technique is regarded as the most sensitive method for strain identification and several bacterial strains have been widely studied using this technique (Kabadjova P X 2002). Use of sequence characterized RAPD fragment is unalternated approach i.e. gaining popularity in diagnosis of bacteria due to is versatility, reliability and sensitivity. Three families of repetitive sequence have been studied in most detailed, including the 35-40 base-pair repetitive extra genomic palindrome (rep) sequence, 124 to 172 base pair enterobacterial repetitive intergenic consensus (ERIC) sequence and the 154 base pair BOX elements (Versalovic J 1994). By calculating the Pearson correlation with the enzyme percentage of all pathways for each pair of organism, transformed the percentage matrix containing the metabolic distance between each pair of organisms from this distance matrix and using the NTSYS programme version 4.0, built a dendrogram using the neighbour-joining (NJ) algorithm. A phylogenetic tree or evolutionary tree is a branching diagram or "tree"

showing the inferred evolutionary relationships among various biological species or other entities based upon similarities and differences in their physical and/or genetic characteristics.

The main objective of the study carried out reveals appearance of bacteria in various study sites specially; agricultural land at five different sampling sites of M.P. The cropping intensity of Madhya Pradesh is 135% and varies from 176% in Harda district (highest) to 108% in Bhind district (lowest). It lies between 21°17′ to 26°52′ N latitude and 74°08′ to 82°49′ E longitude. Crop growth and productivity are determined by a large number of weather, soil and management variables, which vary significantly across space. Remote Sensing (RS) data, acquired repetitively over agricultural land help in identification and mapping of crops and also in assessing crop vigour. As RS data and techniques have improved, the initial efforts that directly related RS-derived vegetation indices (VI) to crop yield have been replaced by approaches that involve retrieved biophysical quantities from RS data (V.K. Dadhwal 2010).

The present study related to microbial isolation of Pseudomonas species from different district of Madhya Pradesh helps to conclude bacterial species similar biochemical property having apprximately common genetic content exhibit molecular diversity to evaluate and compare the new Miniprimer-PCR to the two DNA-based typing techniques and the phenotypic characteristics for the purpose of assessing the diversity of microbial community isolated as collection of fluorescent pseudomonads.

2. Materials and Methods

2.1 Sampling site

Bacterial strains were collected from eight different sites using root free soil, soil surrounding the roots of (Glycine max, Cicer Arietinum, Trigonella foenum graecum, Cajanus Cajan) rhizosphere of different District of Madhya Pradesh, India.

2.2 GIS (Geoinformatic Science)

The procedure adopted in this includes (i) delineation of cropped area using remote sensing (SPOT-Vegetation Index) derived products, (ii) assessment of crop biomass from crop statistics, (iii) estimation of soil carbon stock from point measurement using GIS, (iv) understanding the relationship of crop biomass C with cropping intensity, net irrigated area and nitrogen consumption using regression analysis (Nisha Wani et al 2010).

2.3 Isolation and Identification

Biochemical characterization of isolated colonies was done on the basis of their morphological, cultural and biochemical reactions (Bisen P S and K Verma 1996). All isolates of Pseudomonas were grown in Pikovskaya broth medium and were allowed to grow for 24hrs at 30°C, until rich growth and fully-grown cultures were used for isolation of DNA

2.4 DNA isolation

Isolation of DNA was done using Pseudomonas inoculums of 2.0 O.D., inoculated in 50 ml Pikovaskya broth at 28o ±20C for 24 hours in a shaking incubator. Cell pellet was then obtained by centrifugation at 10,000 rpm (Remi India). Cells were suspended in 25 ml of saline EDTA solution in Erlenmeyer flasks. Lysis of cells were done by addition of 1ml of lysozyme solution at 37°C for 30 min. followed by 25% of 2ml SDS treatment at 600 c for 10 min. It was then allowed to cool at room temperature and 5 ml of 3M sodium acetate was gently mixed to 50ml of 24:1 chloroform. Isoamyl alcohol was added and again centrifuged at 10,000 rpm for 30 min. Out of three layers obtained after centrifugation, the uppermost layer bearing was pipetted out.   two volumes of chilled ethanol was added to it, the white fibrous precipitate at the interface was gently spooled out with the help of glass rod. Isolated DNA was then preserved in tris-EDTA buffer for further study (Maniatis T et al., (1982).

2.5 Quantification of DNA by obtaining the ratio of absorbance at 260nm /280nm

The DNA obtained was quantified at A_{260} for a pure preparation of DNA. Protein content is deduced at A_{280}. A ratio A_{260}/A_{280} up to 1.9 is considered as pure DNA sample. 5μl of DNA sample was taken in a quartz cuvette. The volume was made upto 1.0 ml. With distilled water (995 μl) and absorbance was measured for the solution at wavelength 260nm & 280nm and A260/A280 ratio was calculated. Using the relationship, 1OD at 260nm = 50 μg/ml, the concentration of DNA in the sample was calculated. The DNA is capable of absorbing the UV radiation, and the absorbance ratio i.e., 260nm/280nm indicates the presence and concentration of DNA, the value exceeding more than 1.9 indicates that concentration of protein is more in the isolated sample of DNA.

2.6 The temperature of melting (Tm-value):
The isolated DNA was dissolved in 15 ml of saline citrate. 0.5ml of this solution was dissolved in 4.4ml of saline citrate in four sets of test tubes. Each test tube was then treated at 60˚C, 70˚C, 80˚C and 90˚C for 30 minutes in water bath separately and absorbance was taken at 260nm (Jain, 1998). Values obtained were then plotted and the Tm-value was calculated (Rapley, 1998).

2.7 The determination of %G+C:
The %G+C content (Rapley, 1998) was calculated from the following formula i.e.
    %G+C =2.44 (Tm – 69.4) .28 Where, Tm = Temperature of melting calculated from the graph.

2.8 16S r DNA- RFLP PCR:
Amplification of 16S rDNA was carried out by polymerase chain reaction using a thermo-cycler (Mini Cycler, TM MJ Research PTC-148 BIO-RAD). The PCR were carried out with 50-90 ng of pure genomic DNA. The primers forward and reverse, located respectively, at the extreme 5'and 3' ends of the ribosomal rDNA sequence, enable the amplification of nearly the entire gene. The amplification reactions were performed in a 100 μl volume reaction as shown in (Table.1). The amplified product was run on a 0.8% Agarose gel along with 1Kb MW marker, at a constant voltage and visualized under UV light.

2.9 16Sr DNA-RFLP APMLIFICATION
The amplified 16S rDNA is then digested separately with 05 different restriction enzymes by incubating it over night at 370C.26 Enzyme activity is stopped by providing a low temperature of 4 0C and by adding 2 μl 6x loading buffer to it. Further the enzyme digested PCR product along with 1Kb DNA ladder (Bangalore genie INDIA) in a separate work was estimated by electrophoresis at 55 mV of 2% Agarose gel for four hours. (Table.1)

2.10 RAPD-PCR
Minor modification was used for caring out PCR reaction to produce RAPD profiles. Amplification of DNA fragments was carried out by the Polymerase Chain reaction (Williams JGK 1990) using 30 cycles (Table. 2).

2.11 REP –PCR fingerprinting
The 18 nucleotides in the REP primer make it specific primer for genomic DNA analysis. Both reverse primer: REP 1R (5'-IIIICgICgICATCIggC-3') and forward primer REP 2I (5'-ICgICTTATCIggCCTAC-3') amplifies the complementary sequence found in the genomic DNA varying in size were later analyzed by gel electrophoresis. The master mix was prepared for all the 50 samples as mentioned in the table (table 2.1).The completed PCR reaction is stored at 40C and whole cell REP-PCR products preferably at –200C,to prevent breakdown of the amplified product.

2.12 Genetic Diversity and phylogenic analysis.
The bands originated in a gel as a result of DNA samples was tabulated in the form of matrix (0-1) where 0 indicates absence of band and 1 presence of bands which was then analyzed NTSYS (software clustering of the data which is based on unweighted pair group method with Arithmetic averaging (UPGMA). Similarity was calculating using Jacard Coefficient. Construction of phylogenetic tree was based on the data produced from the clustering and similarity output constructed with the help of 0-1 of the agarose gel with separated DNA bands obtain from various bacterial culture.

3. Result and Discussion

3.1 Sampling sites
The study was conducted by obtaining various soil samples in order to perform identification of agriculturally important microbial species. Some of the districts Bhopal ,Hoshangabad, Betul, Harda, Gwalior, Shahdol   and Raisen of Madhya Pradesh were identified for soil sampling.

3.2 GIS (Geoinformatic Science)
Agricultural area map of Madhya Pradesh includes total geographical area of the state is 30.8 M ha, of which total net sown area in 2005-06 was 14.9 M ha (48.5%), net irrigated area is 5.5 M ha (36.9% of agricultural area) and total forest area is   8.68 M ha (DES 2006). District-wise agriculture area distribution was delineated from the base map Guna and Umaria districts had the highest (6337 km2) and lowest net sown area (1109 km2),

respectively which was attributed to extent of geographical area and forest cover. Among all other districts of Madhya Pradesh, Hoshangabad had the maximum net irrigated area (79.2% of net sown area) due to the availability of an irrigation canal and Dindori had the minimum net irrigated area (0.40%) as the district was mostly covered with forest and mountainous terrain. The majority of the crops in Madhya Pradesh were rainfed and only 36.9% of the net sown area was under irrigation (Nisha Wani 2010).

3.3 Isolation and Identification

Soil profile of Madhya Pradesh shows appearances of black cotton soil in texture with alkaline property. Microbial cultures were obtained after incubation at 24^0C on synthetic Nutrient agar medium showed round slimy, colonies which were further characterized Shiny, pinpoint colonies with smooth margin have raised elevation and showed yellowish pigment, although sometime pigments were not prominent conforming mixture of species of Pseudomonas. Dilution studies confirm dot like circular colonies when cells were observed under contrast illumination. Similarly, when cells were grown on King's B medium after 48 hrs incubation at 30^oC pigmentation reflected the occurrence of Pseudomonas fluorescence on culture plates. Cells grown on nutrient agar medium once inoculated on Pikovaskay's media zone of phosphate solubilization were seen after 20hrs of incubation with gradual increase in diameter.

3.4 DNA Isolation Quantification Tm value and G+C % content

The strand of double helix DNA can be separated by heating a process generally called melting. The double stranded molecules show lower ultraviolet absorbance than single stranded molecules. Therefore if the ultraviolet absorbance of a nucleic acid solution is measured while it is being heated, the increase in absorbance when the double stranded molecule are converted to single stranded molecule will show the temperature at which strand separation occurs. The mid point of the transition, called Tm is a function of the GC content of the DNA. AT and GC pairs are held together by two and three hydrogen bonds, respectively so high temperature is required to disrupt GC pair. For this reason, the value of Tm is related to the base composition of the DNA, and in the solution is standardized with respect to salt concentration and pH, Tm value can be used to measure the base composition.

The base sequence of G+C % contents is known to resist the exposure to mutagen or any hazardous chemicals. In the present observation G+C content in the present concentration is expressed in (Table 3) with respective Tm value. Mostly, strains S-2, S-10, S-21, S-28, S-37, S-47, showed 61.0% G+C content. However, lowest value of % G+C content of isolated bacterial strains observed was 58. 80%. Strains S-3, S-11, S-22, S-29,S-37 AND S-48 were found to have 58.8 % G+C content.

In all the 50 strains isolated Tm value range between 93.7 to 94.5. Narrow range of variation between all isolated strains 1 to 50 shows closed similarity between Pseudomonas species isolated during course of study. In order to identify Pseudomonas spp isolated from different samples of soil, genomic analysis have been performed. Microbial diversity is based upon variation in organization of genomic content. Study of microbial diversity is a need of microbial ecological research .The production of accurate variation in genomic content is dependent upon external factors. Although, the genus shows internal variation in relation to pattern of appearance of genomic content. DNA sample of 50 isolated strains were taken using Helms method (Helms et. al., 1985; Tracy, 1981; Mainatis et. al., 1982).Gel was performed at 25^0C with 0.8% agaorse. Each of the 50 species included in Pseudomonas genus showed ordinal profile of DNA content (Fig 1).

3.5 PCR Amplification

The objective of the present investigation was to study the genetic and functional diversity of phosphate solubilizing fluorescent Pseudomonads associated with rhizospheric soils of soyabean and other symbiotic and free-living microorganism by an array of in vitro assays, gene amplification techniques, fermentation methods and chromatographic analyses.

Phosphorus Solubilizing bacteria known as fluorescent Pseudomonads often associated with rhizosphere are known to synthesize phytohormone and are known to suppress the growth of phytopathogens. These groups of bacteria exhibit multiple functional traits such as solubilizing of inorganic phosphate and iron production, production of vitamin, phytohormone and antimicrobial metabolites. In the present investigation out of 50 isolates most of the Pseudomonas species have been identified as phosphate solubilizer. 16SrDNA of 50 isolated strains of pseudomonas were PCR amplified with Universal Primer P. aeuroginosa, P. fluorescence, P.Putida, P. Streuzi. The sequence of ribosomal DNA is known to determine phylogeny of microbes as well as higher organism the rate of mutation frequency of these conserved sequence is extremely low. Therefore, most of the species reflects, common pattern as they are conserved in nature. Restriction enzyme known for PCR analysis of

16SrDNA segments varies, from species to species although for present study Hae III restriction enzyme was preferred in case of 16SrDNA segment of Pseudomonas species (Fig .2).

The bands resulting after PCR reaction were seen on agarose gel. Use of Hind III, Mbo I, Taq I, results variation in pattern of appearance of bands when DNA is subjected to digestion. Bands appearance on acryl amide gel after digestion falls within 200-800bp. The DNA band patterns appearing on gel were further subjected to computational analysis using UPGMA profile the similarity index were subjected to analysis on NTSYS software using unweighted pair method. The results appearing using restriction enzymes treatment is illustrated (Fig 3).

Dendrogram were prepared using coefficient scale shows point of divergent at 68% of similarity. Second measure growth of pseudomonas preferred with genetic relationship at 80% similarity (Fig 3) strains S-6, S-15, S-16, S-19 and S-20 shows 20% dissimilarity among each other. However, a smaller group shows divergent at 87% similarity and shows only one species of Pseudomonas i.e S-3.Strains of Pseudomonas S-5 and S-14 shows dissimilarity of 7% i.e. they are much similar among each other. Strain S-36 is similar to S-3,S-33,S-34,S-45,S-35,S-48,S-46 and shows similarity of 87%. However, strain S-3, shows only 4% dissimilarity to rest of the group. Minor deviation in similarity among all isolates of Pseudomonas shows there mutational competency and resistance towards strains .The bands appearing during 16SrDNA restriction enzyme treatment the level of divergent in base substitution during course of evolution. The phylogenetic distance between species i.e. S-1 to S-50 of Pseudomonas the presence variation and arrangement of nucleotide sequence as supported by appearance of dendrogram (Fig.3).

The dissimilarity among base pair also affects the reading frame of organism. Pseudomonas species isolated were subjected to random amplification to observe polymorphic pattern of DNA. The reaction mixture were examined for band profile on 2% agarose gel (Fig 3.4). The bands appeared expressed pattern of banding between 200 to 900 bp observations of RAPD were subjected to NTSYS program for reproduction of phylogeny (Fig 5).

RAPD PCR of isolated strains of Pseudomonas represented high degree of variability. Strain S-4, S-10 and strains S-42, S-44, showed dissimilarity of 20% with a narrow range of bifurcation, strain S-43 an S-44 shows 100% similarity. The similarity index with the help of RAPD PCR of Pseudomonas species isolates divergent only with 15% of similarity index maximum similarity of appeared at 80%. Heterogeneity and variation with in species S-27 and S-38 shows 67% similarity strains S-26 shows 77% dissimilarity with rest of the strain Pseudomonas isolated polymorphism represented repeated appearance of common sequence and in the present of observation occurrence of such bands for more prevalent . The site of action of enzyme most preferably more available thus, Thus, by implementing Jaccard's similarity coefficient and UPGMA cluster method minute degree of deviation resultant hieracy of polymorphism. Furthermore, standard PCR technique of REP-PCR was implemented and reproduced to show banding pattern as shown in (Fig .5).

The size of DNA fragment using REP-PCR primer range between 200 to 1000 bp. All the 50 isolates of Pseudomonas from different soils of Madhya Pradesh although having approximately similar G+C % content and Tm value represented variation were subjected to REP-PCR fingerprinting tools .

Purified DNA was subjected to digestion using forward and reversed primer REP 1R (5'-IIIICgICgICATCIggC-3') and REP 2I (5'-ICgICTTATCIggCCTAC-3') and illustrate in (Fig .5). Three distinct clusters were found with 55%, 60%, and 65 % respectively S-13,S-14,S-15,S-15,S-38,S-16,S-38,S-39 S-40,S-41, and S-46 falls with 65% similarity. However, strains S-5, S-8, S-21, S-24, S-30 and S-33 shows 60 % similarity amongst each other.   Strain S-51 S2 S3 S26 S27 S28 differs with these 5 % to s-25.

Genomic observation used as tool to study differential appearance and relationship amongst Pseudomonads species. Molecular techniques offer advantage over biochemical parameters as genome resultants any metabolic process, thus, maintaining continuity of inheritance .All the 50 strains of pseudomonas sp such as Pseudomonas aeruginosa, P.putida, P. fluroscence, and P. streuzi were shown to have genetic similarity with variation in arrangement of nucleotides in genome. Ornamentation of genome represent base substitution thus defining evolutionary distance along with constantly of character within species.

4.Conclution

The phosphate solubilizing organisms are efficient in releasing phosphorus from inorganic and organic form of phosphrous by solubilization and mineralization mechanism. So it is necessary to isolate the efficient phosphate solubilizer bacteria. In present study phosphate solubilizer Pseudomonas species were isolated from agricultural land of M.P. and their genetic characterization was done by using molecular techniques. PCR based method resolve genetic variation between different microbial species and strains. In this study 16s rDNA-RFLP analysis ERIC-REP, BOX PCR were used for identification and characterization of isolated strains.

The present research helps in conserve biodiversity with particular sequences of phosphate solubilizing microbes and provides opportunities to increasing agricultural production. This research solves the specific problem of identification, characterization, detection and monitoring of agricultural important isolates/strains. The isolated were efficient bioinoculants to the farmers and will be used for improvement of crop productivity.

Taxonomic affiliation of bacteria was done on the basis of 16S rDNA gene similarity and molecular phylogenetic analyses. Organism Pseudomonas species isolated were subjected to random amplification to observe polymorphic pattern of DNA. Polymorphism is a natural phenomena which helps to study similarity amongst species using defined primers appearance of repeated sequence of a bases in pseudomonas genus results banding pattern after performing PCR reaction 16SrDNA of 50 isolated strains of pseudomonas were PCR amplified with Universal Primer P. aeuroginosa, P. fluorescence, P.Putida, P. Streuzi. The similarity index with the help of RAPD PCR of Pseudomonas species isolates divergent only with 15% of similarity index maximum similarity of appeared at 80%.

Heterogeneity and variation with in species S-27 and S-38 shows 67% similarity strains S-26 shows 77% dissimilarity with rest of the strain Pseudomonas isolated polymorphism represented repeated appearance of common sequence and in the present observation occurrence of such bands is far more prevalent. The size of DNA fragment using REP-PCR primer range between 200 to 1000 bp.

All the 50 strains of pseudomonas sp such as Pseudomonas aeruginosa, P.putida, P. fluroscence, and P. streuzi were shown to have genetic similarity with variation in arrangement of nucleotides in genome. Expression of species within the genus in regulated but reading frame of organism, therefore, with common concentration of DNA most of the species shows architectural motive once NTSYS prediction were made using UPGMA .Ornamentation of genome represent base substitution thus defining evolutionary distance along with constantly of character within species. All the Pseudomonas isolated looking into their biochemical profile can be used as PGPR and biocontrol agents.

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Table 1. The PCR reaction mix for a 100μl reaction mixture was as follows

S.No.	Component	Concentration
1.	PCR buffer (10x)	10.00μl
2.	dNTP mix (10mM)	1.0μl
3	MgCl2 (50mM)	6.0μl
4.	Primer PA (100ng/μl)	1.0μl
5.	Primer PH (100ng/μl)	1.0μl
6	Tag DNA Polymerase(1.5	0.5μl
7.	Template DNA (50ng)	10.0μl
8.	MilliQ water	70.5μl
	Total	100.0μl

Table 2. PCR cycle for amplification of 16S rDNA

Amplification stage	Temperature (^{0}C)	Time
Initial Denaturation	94	5min
Denaturation	94 (30cycle)	30 sec.
Annealing	50 (30cycle)	40sec.
Extension	72 (30cycle)	90sec
Final extension	72(30cycle)	7 min.

Table 3. Primers used for amplification of 16s rDNA region.

S.No.	Primer	Sequence
1.	Reverse	**(5' CCG AAT TCG TCG ACA ACA GAG-3')**
2.	Forward	**(5'-CCC GGG ATC CAA GCT TAA G -3')**

Table 4. Restriction Enzymes

S.No.	Enzyme	Sequence	Assays Temp.
1.	Alu I	AG↓CT	37^0c
2..	Mbo I	↓GATC	37^0c
3.	Hind III	A↓AGCTT	37^0c
4.	Hae III	GG↓CC	37^0c
5.	Taq I	T↓CGA	37^0c

Table 5. Primers Used for RAPD analysis.

S.No	Primer Name	Primer Sequence
1	OPK-10	5'-GTGCAACGTG-3'
2	OPK-20	5'-GTGTCGCGAG-3'
3	OPK-15	5'-ACGCACAACC-3'
4	OPK-13	5'-GGTTGTACCC-3'
5	OPK-11	5'-AATGCCCCAG-3'

Table 6. PCR programmed for REP Analysis

Amplification stag	Temperature (^{0}c)	Time
Initial Denaturation	95	7min
Denaturation	94 (35cycle)	1min
Annealing	40 (30cycle)	1min
Extension	72 (30cycle)	1min
Final extension	72(30cycle)	16 min.
Final hold	4^0C for 0 sec.	
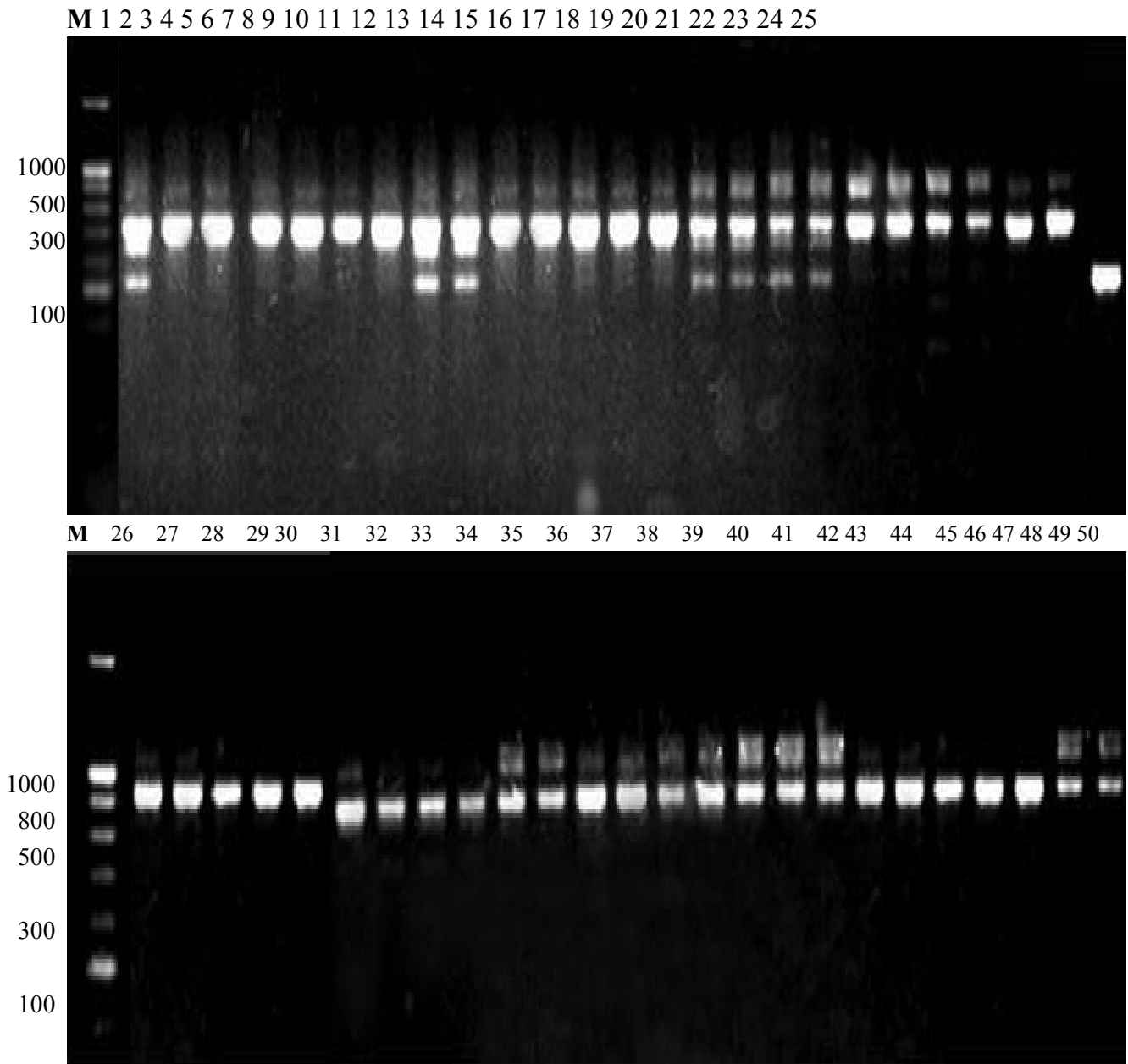
FIG. 1: Purity of DNA of Pseudomonas species isolated from agricultural field of M.P. on 0.8% agarose gel.
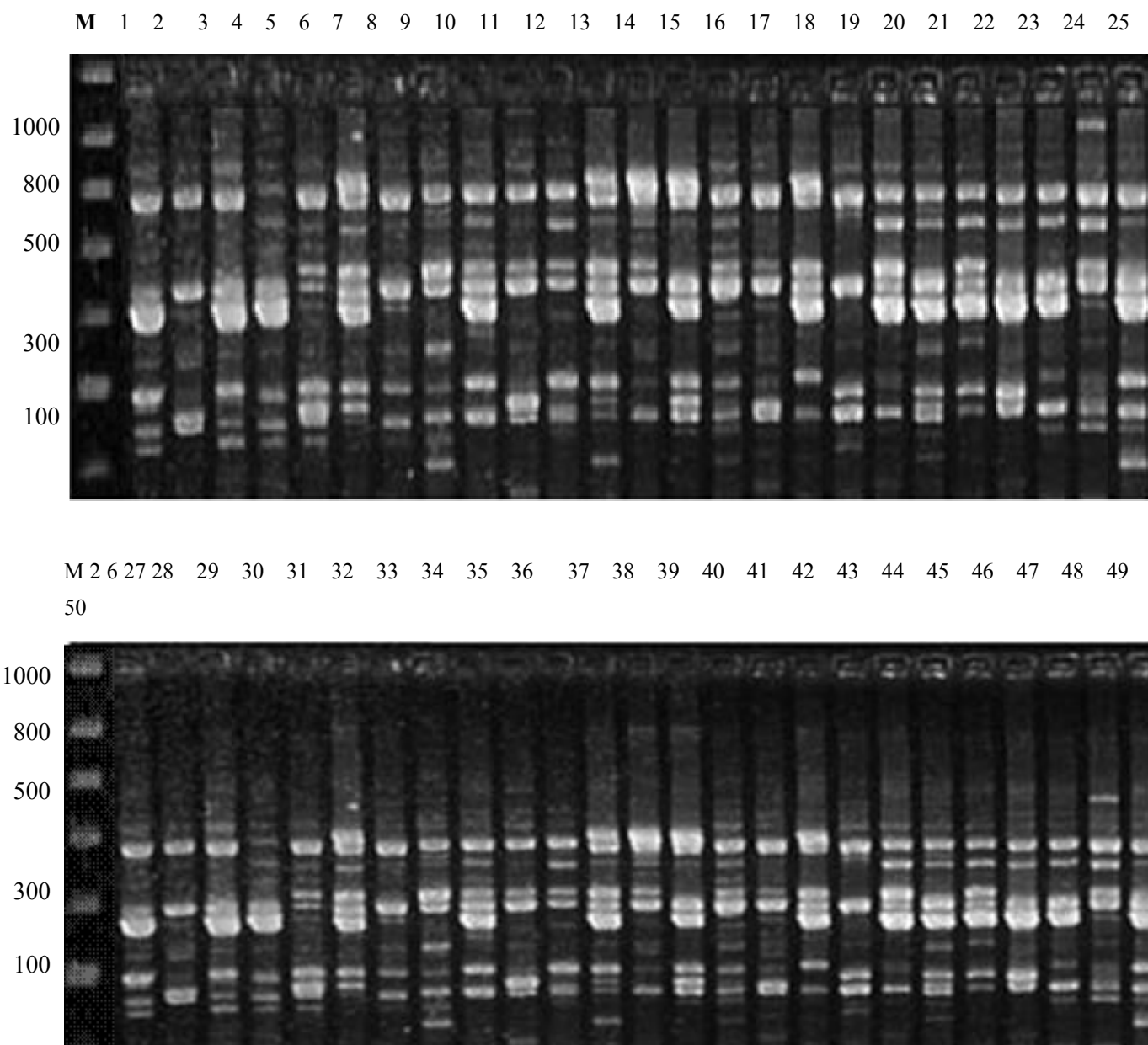
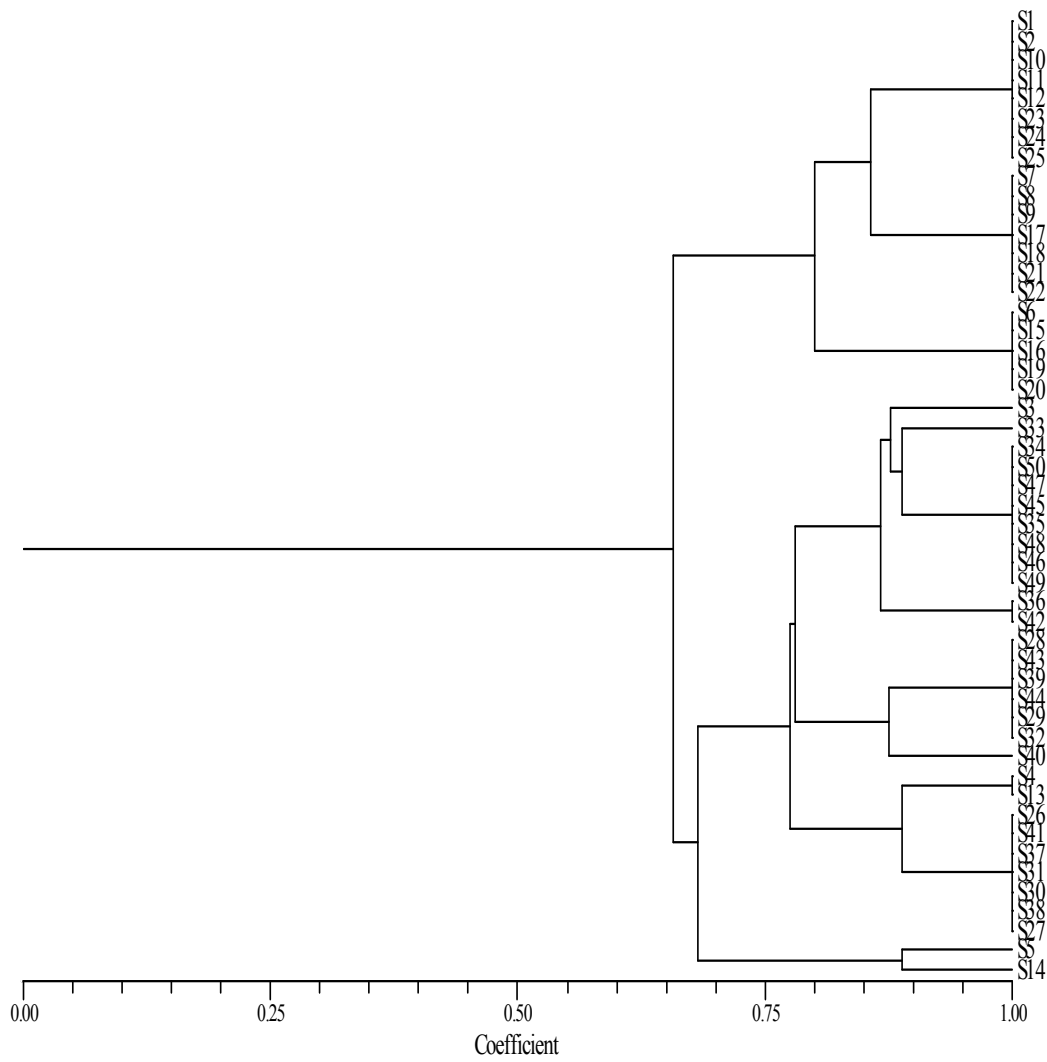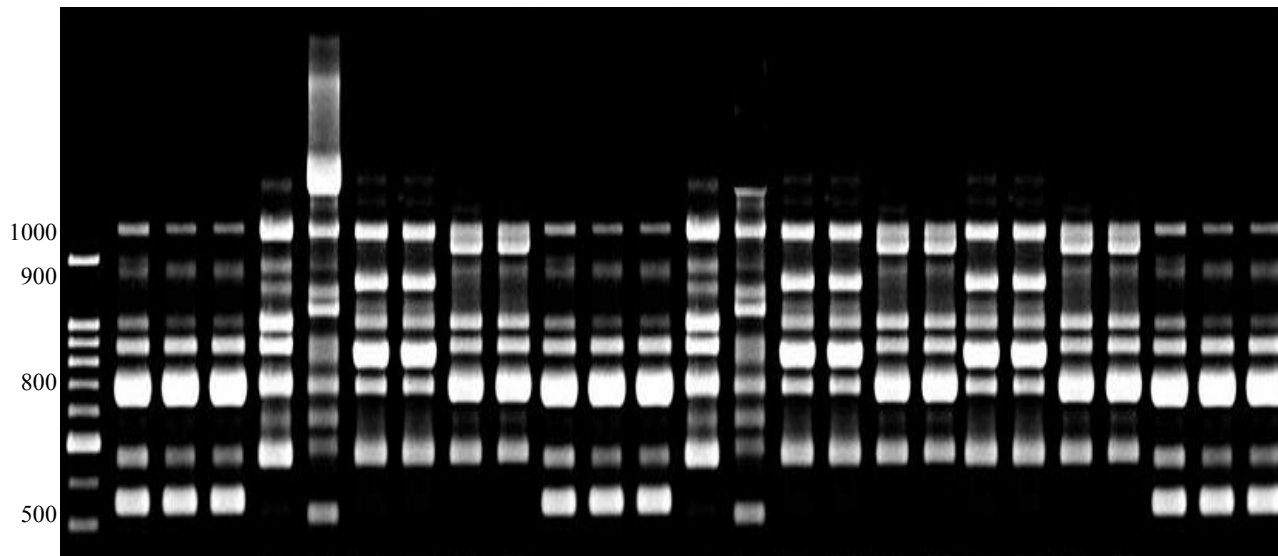Fig.2: Restriction enzyme Hae III treated 16S rDNA segments of isolated strains of Pseudomonas sp. On 2% agrose gel.
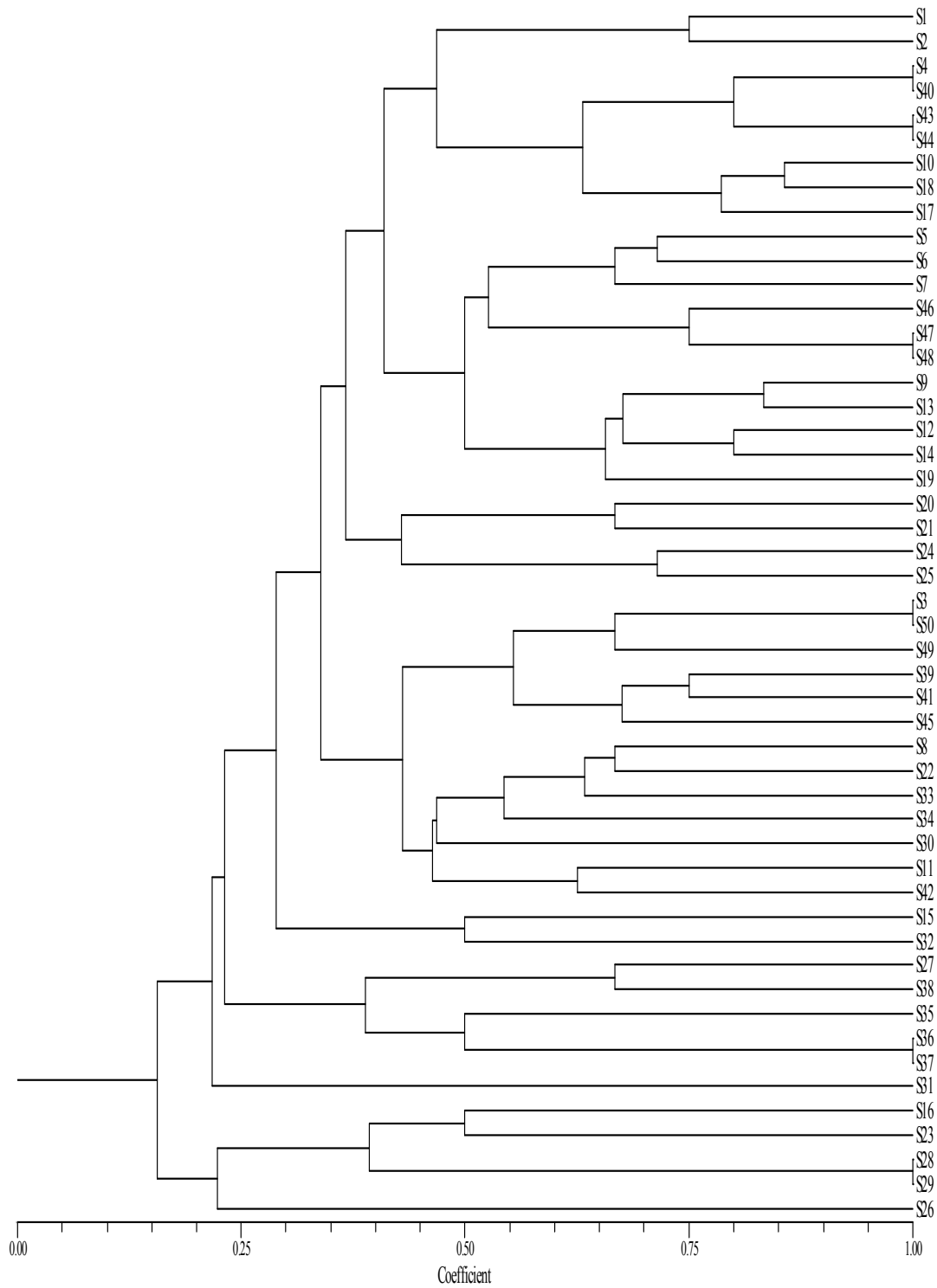
Fig.3: Dendrogram based on Hae III treated 16S rDNA segment of Pseudomonas isolates showing genetic relatedness using Jaccord's similarity coefficient and UPGMA cluster method.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
24 25

M 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46
47 48 49 50
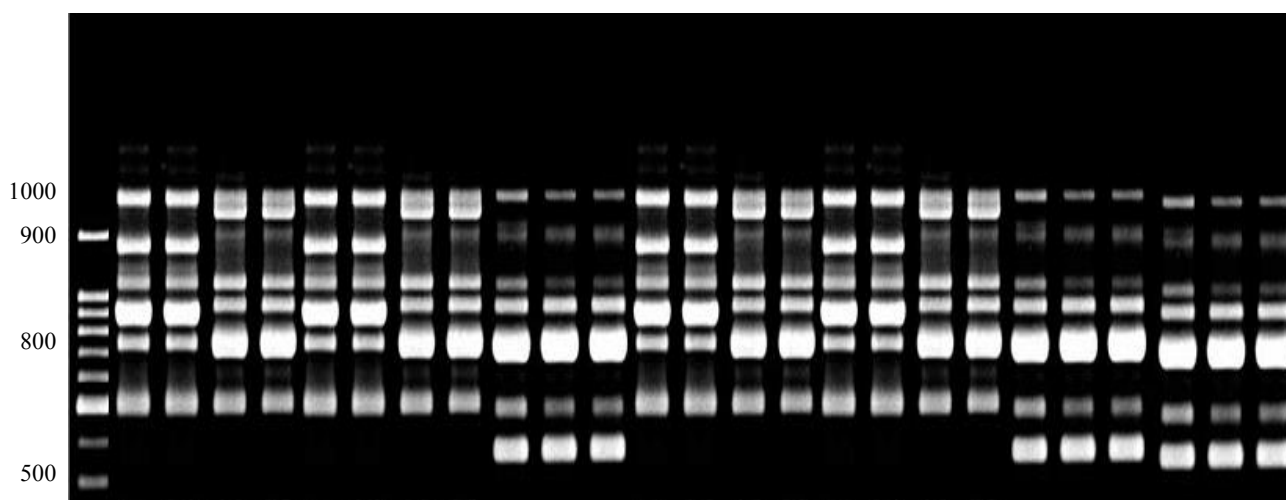
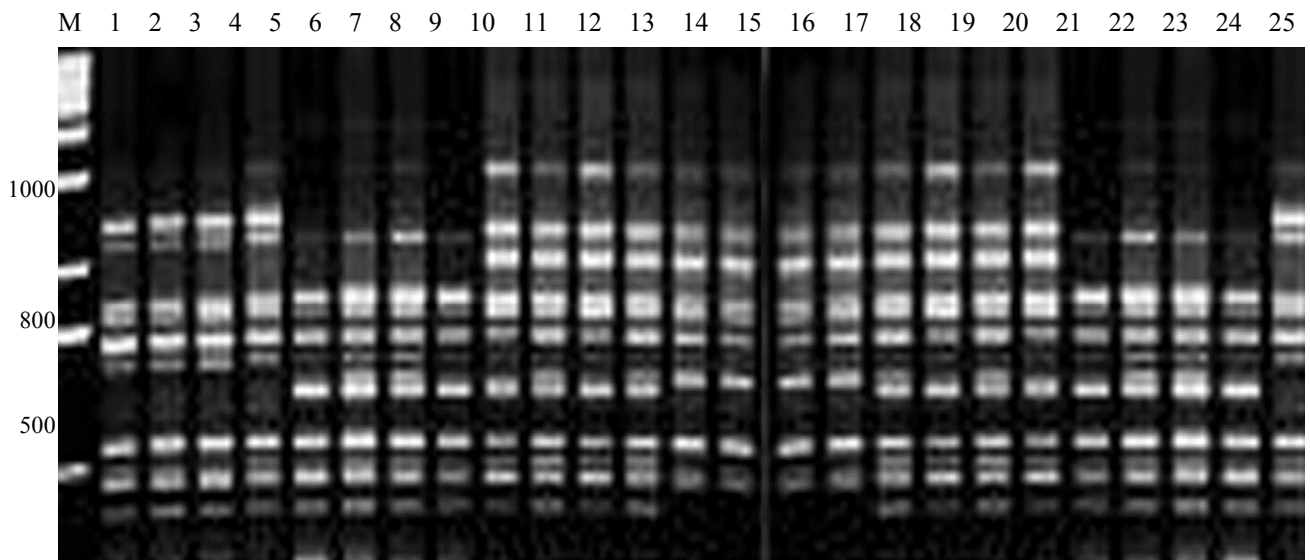Fig.4: RAPD-PCR fingerprinting of Pseudomonas strains with OPK20 primer isolated from agricultural field of M. P. on 2% agrose gel

Fig.5: Dendrogram based on RAPD-PCR of Pseudomonas   isolates showing genetic relatedness using Jaccord's similarity coefficient and UPGMA cluster method.

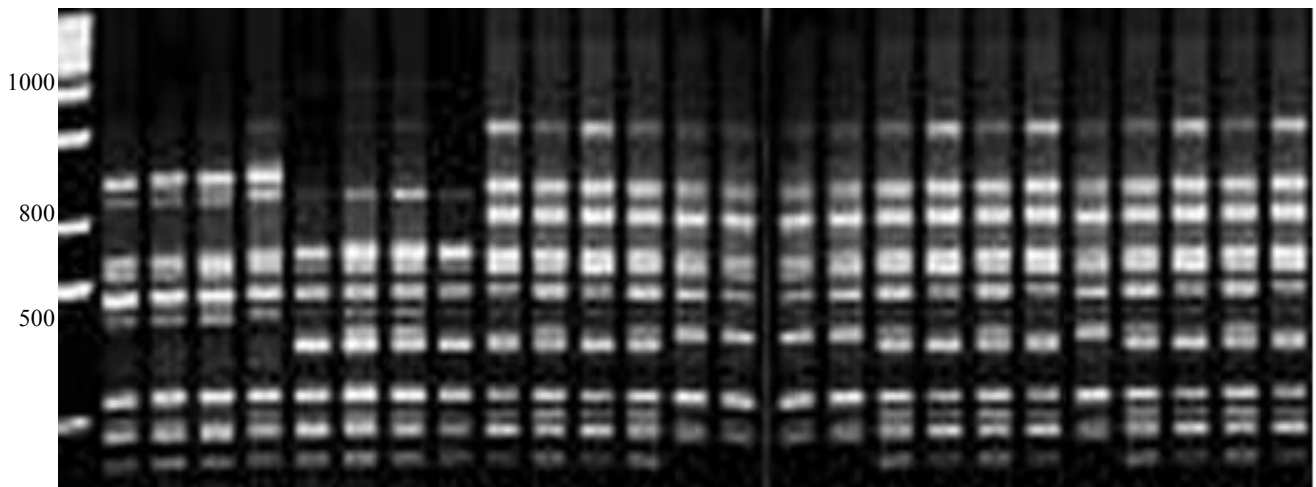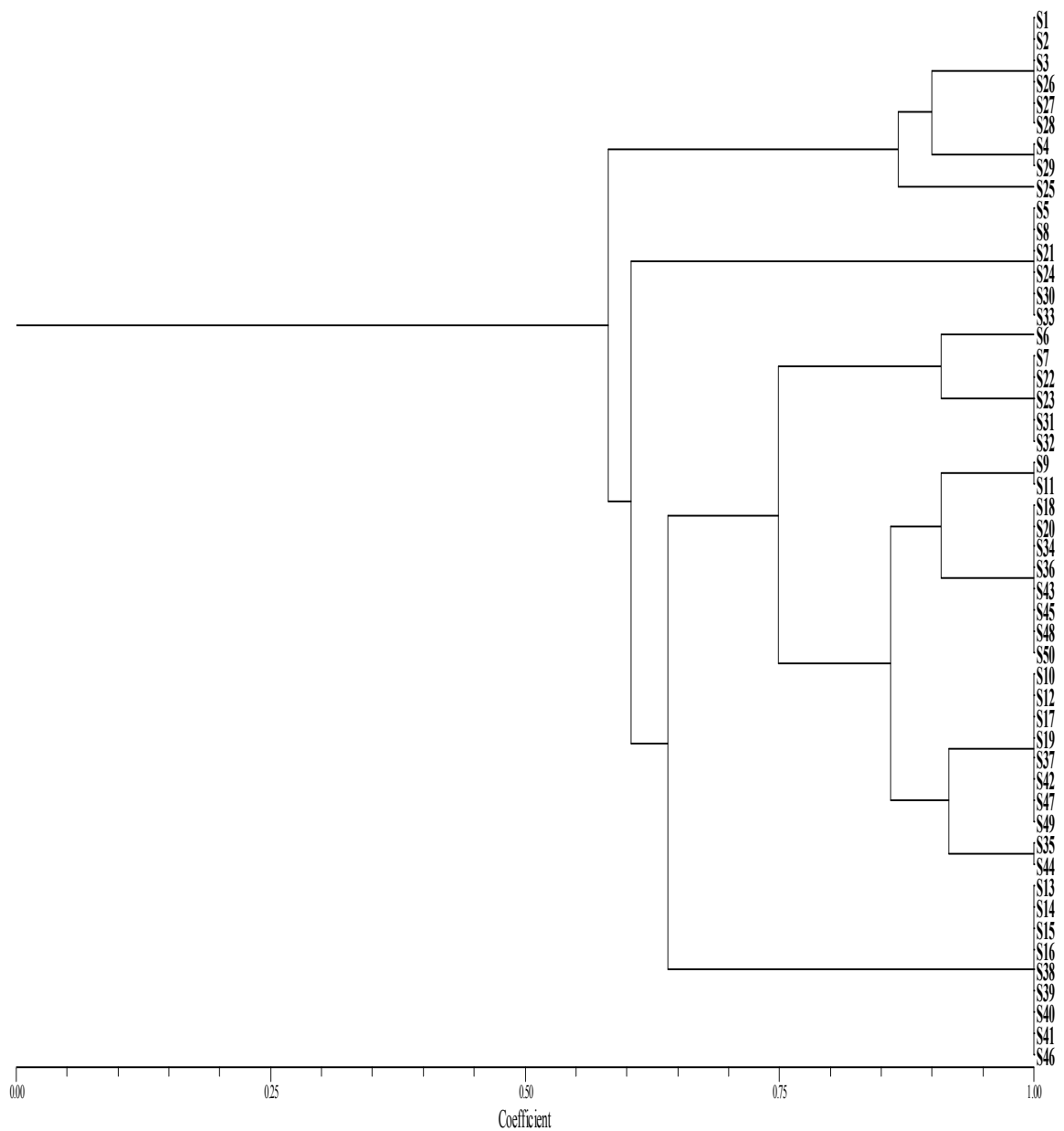Fig.6 : REP-PCR fingerprinting of Pseudomonas strains isolated from agricultural field of M. P.   on 2% agrose gel

Fig.7 : Dendrogram based on REP-PCR of Pseudomonas isolates showing genetic relatedness using Jaccord's similarity coefficient and UPGMA cluster method.

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