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RESEARCH ARTICLE

MOLECULAR IDENTIFICATION OF TIMBER DECAY FUNGI OF GUJARAT, INDIA BY RAPD

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ABSTRACT

Several molecular methods for detecting wood decay fungi have been developed, but methods for direct extraction of fungal DNA and fungal species identification during the incipient stage of decay could benefit to save the timber from decay. Timber degrading fungi was collected from different areas of Gujarat. The samples were brought to the Lab for their DNA isolation and quantification as well. Random amplified polymorphic DNA analysis was used to determine the % variability in the genomic profiles of *Lenites sterioides, L. betulina, L. exima, Flavodon flavus* strain 1, *F. flavus* strain 2, *Ganoderma lucidum, G. applanatum, Phelinus robustus, Schizophyllum commune, Pluerotus pulmonarius*, and *Stereum hirsutum*. Initially 18 RAPD primers (20 primers Kit E, IDT USA; 160 primers, kit- J, K, L, N, O, P, Q, R, Operon technologies Inc., USA) were screened and out of which 15 primers responded with minimum 6 loci (bands) were included in the study. 10 primers were screened for the amplification of DNA fragments. 100 percent polymorphism was observed when primers OPL-1, OPL-5, OPO-7, OPL-14, OPL-18, OPN-10, OPL-4, and OPQ-15. Dendrogram of the RAPD analysis provided information of the genetic variability among the timber degrading fungi. Over all PP among seven genera through RAPD was found to be 98.81 with 2 markers common among all genuses studied. In the pair wise comparison the mean PP was 98.87.

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INTRODUCTION

Basidiomycetous members are mainly classified on the morphology of fruit bodies. However, many strains of Basidiomycete species do not form fruit bodies readily at an early decay conditions. The development of a simple method for distinguishing species or strains with vegetative mycelia, therefore, is important. So, recently molecular markers, such as isozymes Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) have been used to detect genetic differences in the species and strains of basidiomycetous. Among these molecular markers, RAPD which was introduced by the use of polymerase chain reaction (PCR) with arbitory 10 mer primers. These primers are probably able to find distinct complementary sequences in the genome producing specific banding patterns. The resulting PCR fragments are then separated by electrophoresis to obtain fingerprints that may distinguish fungal species varieties or strains (Williams *et al*., 1990). Some of the specific DNA fragments detected in a profile may be cut out of the gel and sequenced to obtain a SCAR (Sequence-characterized amplified region), into which specific primers can be designed for a more precise PCR detection. It can express DNA variations for distinguishing basidiomycetes species and strains with less labor and high reliability (Ito *et al*., 1998). RAPD results are also useful for the analysis of the genetic diversity among populations. Fingerprints are scored for the presence (1) or absence (0) of bands of various molecular weight sizes in the form of binary matrices. Data are analyzed to obtain statistic coefficients among the isolates that are then clustered to generate dendrograms. RAPDs have been used to analyze the genetic diversity among different species and races of Fusarium spp. (Arici and Koc, 2010) RAPD is especially useful for analyzing a large number of marker loci and this method was used for the construction of genetic maps in filamentous fungi also (Williams *et al*., 1990; Xu and Leslie 1996; Kerrigan *et al*., 1993). Two genetic maps have been reported for Lentinula edodes using RAPD Markers (Kwan and Xu, 2002). Ten pure cultures of different genera of the aphyllophorales were molecularly characterized by using RAPD. In the present paper Timber degrading fungi like Lenites sterioides, L. betulina, L. exima, F. flavus strain 1, F.

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flavus strain 2, G. lucidum, G. applanatum, P. robustus, *S. commune, P. pulmonarius*, and *S. hirsutum* were identified by using molecular techniques.

MATERIALS AND METHODS

Isolation of Fungi

The fungi associated with the samples were isolated. Wood chips measuring 5 mm × 5 mm × 2 mm were aseptically removed from the samples and transferred to petriplates containing 2 different cultural media: 2% malt extract agar and PDA medium amended with 250µg Streptomycin sulphate per ml. The first medium was intended to isolate Basidiomycetous fungi and the second medium to specifically isolate total fungi. Eight pieces of wood were removed from each sample and placed in 2 petriplates. These plates were incubated at 25±2°C for 7 days. Once fungal colonies were formed in the agar plates, each colony was transferred to a new agar slant to obtain a pure culture.

Genomic DNA Extraction

Genomic DNA was extracted from freshly grown culture of fungal mycelium on MEA plates and extraction was carried out in two phases as described below:

Solutions for extraction

Tris saturated phenol, phenol:chloroform:isoamyl alcohol (25:24:1), chloroform:isoamyl alcohol (24:1), 70% and 80% ethanol, 5 M NaCl, 3 M sodium acetate (pH 5.2) and TE buffer (10mM Tris-HCl, 1 mM EDTA, pH 8.0). Solutions and buffers prepared were autoclaved at 121 ºC temperature and 15 psi pressure (Tommy autoclave, Japan). The stock solution of RNase 10 mg/ml was prepared freshly as per the user manual (Sigma, USA).

Extraction phase

0.1 g of mycelial mat was grinded in a pre-cooled mortar with pestle to a fine powder using liquid nitrogen along with 10 mg (2% of extraction buffer) of PVP (Sigma, USA). The powdered tissue was scraped into a 2.0 mL microcentrifuge tube containing pre-heated (65 ºC) extraction buffer in 1:5 ratio (0.5 mL). β-mercaptoethanol was added to the final concentration of 0.2 M and mixed well. The mixture was incubated in water bath at 65 ºC for 90 min and cooled for 5 min. An equal volume of chloroform:isoamyl alcohol mixture (24:1) was added to the extract and mixed by gentle inversion for 5 to 10 min to form a uniform emulsion. The mixture was centrifuged at 8000 rpm for 8 min at RT. Chloroform: isoamyl alcohol extraction step was done twice. The aqueous phase was pipetted out gently, avoiding the interface. To the above solution, 5 M NaCl (to final concentration 2 M) and 0.6 volumes (V/V) isopropanol was added and incubated at RT for 1 h. Two volumes of 80% ethanol was added to the above solution and incubated again for 10 min at RT for DNA precipitation. After incubation the mixture was centrifuged at 10,000 rpm for 15 min at RT. The white/translucent pellet was washed with 70% ethanol, dried and resuspended in 200 µl of TE buffer.

Purification phase

The sample was treated with RNase (10 µL of 10 mg/mL of RNase) and incubated at 37 ºC for 60 min. After incubation with RNase, one volume of Tris saturated phenol (pH 8.0) was added and mixed gently by inverting the micro-centrifuge tube till it formed a milky white emulsion. The emulsion was then centrifuged at 10,000 rpm for 5 min at RT. The supernatant was pipetted out into a fresh tube. The sample was then extracted with equal volume of chloroform: isoamyl alcohol (24:1) twice. The DNA was reprecipitated with 0.6 volumes of isopropanol, 2.0 M NaCl (final concentration) and incubated for 10 min. To the above solution, 20 µl of sodium acetate and 1 volume of 80% ethanol was added, incubated at RT for 30 min, and centrifuged at 10,000 rpm for 15 min to pelletout the DNA. The pellet was then washed with 70% ethanol twice; air dried and finally suspended in 40-50 µl of TE buffer.

Quantification of Genomic DNA

After extraction of genomic DNA, quantification was done according to Sambrook *et al.* (1982). 15 µl of extracted DNA was dissolved in 735 µl of TE buffer and O.D. was taken at 260 and 280 nm (using CARY 500 scan UV visible spectrophotometer).

Quantity of DNA was calculated by using following formula:
Amount of DNA (ng/µl) = O.D. at 260 X dilution factor X 50 (extension coefficient)

Quality was assessed by taking the (O.D. at 260)/(O.D. at 280). Samples which gave the O.D. between 1.6-1.8 were used in further work.

Restriction Digestion

The extracted genomic DNA was digested by incubating with *Eco*RI, *Mse*I restriction endonucleases along with control (without adding enzyme) in the corresponding buffers at 37 ºC for 3 h according to the users manual. Digested DNA along with control was analyzed by running the samples in 1.2% agarose gel at 50 V and stained with ethidium bromide.

Polymerase Chain Reaction (PCR)

PCR was carried out as per the required volume with stock concentrations of reaction buffer (10X), $MgCl_2$ (25 mM), dNTPs, Taq DNA Polymerase (5 U/µl) (Bioenzyme, USA) and, template DNA (250 ng/µl). The reaction was carried out in Thermal cycler (Eppendorf ep gradient S) Final concentration of PCR reagents in reaction mixture (100 µl) are Taq polymerase 5 U, 1X reaction buffer, 3.5 mM $MgCl_2$, 800 µM dNTPs, 0.4-1 µM of each Primer, 50-100 ng Template DNA

Reaction was carried out as per the program given bellow:

Step	Temperature	Time
Initial denaturation	94 ºC	3 min
Denaturation	94 ºC	30 sec
Annealing	----	30 sec
Extension	72 ºC	----
Final extension	72 ºC	5 min

Agarose Gel Electrophoresis

Plate preparation and casting the gels: Cleaned agarose gel casting cassette and comb were wiped with methanol. The

open sides of the tray were sealed with gel sealing tape. The comb was placed in the given slits of the plate. Calculated amount of agarose in TBE buffer was mixed to prepare 1.5% solution. The agarose was dissolved completely in the buffer by heating the mixture at 80-85 ºC in microwave oven and was cooled to 50 ºC. Liquid was gently poured into the casting tray before it gets solidified. The combs and sealed tape were removed slowly after complete solidification of the agarose gel.

Preparation of Samples and Scanning of Gels: The amplified DNA samples having approximately 15 µl volume were mixed with 4 µl gel loading dye and were carefully loaded in the wells using gel loading tips. Electrophoresis was carried out at 100-50V. The gel was stained by ethidium bromide solution having of 0.1mg/ml concentration for 15-20 min. The gel images were recorded in JPEG or TIF formats using gel documentation system (Syngene, USA). The gels were analyzed by using the software Gene Tool (Syngene, USA).

Random Amplified Polymorphic DNA (RAPD) Analysis: Amplification of RAPD fragments was performed according to Williams *et al.* (1990) using decamer arbitrary primers (Operon technologies Inc, USA; IDT, USA). The reaction was carried out in a volume of 25 µl of reaction mixture containing final concentration of 10 mM Tris-HCl (pH 9.0), 50mM KCl, 0.1% Triton X-100, 0.2 mM dNTPs, 3.0 mM MgCl2, 0.4µM primer, 25 ng template, 1unit Taq DNA polymerase (Sigma, USA). Amplification was performed in programmed thermal cycler with a program of initial denaturation at 94 ºC for 3 min, 42 cycles of denaturation at 94 ºC for 30 sec, primer annealing at 32 ºC for 1min, extension at 72 ºC for 2.5 min, and final extension at 72 ºC for 4 min. amplification products were electrophoresed in 1.5% TBE. The gels were stained with ethidium bromide and documented using gel documentation system (Syngene, UK). Experiment with each primer was done three times those primers gave reproducible fingerprints were considered for data analysis.

RAPD Data Analysis

Acquired RAPD finger prints were statistically analyzed with following assumptions.

1) The populations are in hardy Weinberg equilibrium.
2) Each band represents the phenotype at a single biallelic distinct locus.
3) Comigrating band represent homologous loci.
4) Polymorphic loci are inherited in a Mendelian fashion.

Fragment sizes were designated as loci, and were considered as Biallelic (present = 1, absent = 0) and made the binary matrix. Only those loci amplified strongly in each instance with reproducibility were scored and included in the analyses ignoring the intensity of the bands.

Genetic similarity was calculated according to the following formula:

$$F = \frac{2Nxy}{(Nx+Ny)}$$

Nxy = the number of bands shared by two species.
Nx and Ny = are the number of fragments in each sample.
Genetic disparity was calculated by formula.

$$P=1-F$$

Percentage of polymorphism was calculated by using following formula:

$$\text{Percentage of Polymorphism} = \frac{\text{No. of Polymorphic Bands}}{\text{Total number of Bands}} \times 100$$

Phylogenetic trees were constructed according to Jaccard (1908) using binary data generated by RAPD and AFLP excluding the intraspecific polymorphic markers, followed by bootstrapping analysis across the loci (Felsenstein, 1985) with the help of statistical analysis software NTSYS-pc version 2.11f.

RESULTS AND DISCUSSION

Isolation of Fungi

The fungi associated with the wood samples were isolated. Around 10 wood decay fungi were isolated on Malt extract agar medium. The growth of fungi was much better in Malt extract medium. They were identified by using traditional method *L. sterioides, L. betulina, L. exima, F. flavus* strain 1, *F. flavus* strain 2, *G. lucidum, G. applanatum, P. robustus, S. commune, P. pulmonarius*, and *S. hirsutum* (Plate I Fig. A).

Genomic DNA Extraction and Quantification

The Genomic DNA was extracted from timber decay fungi like *L. sterioides, L. betulina, L. exima, F. flavus* strain 1, *F. flavus* strain 2, *G. lucidum, G. applanatum, P. robustus, S. commune, P. pulmonarius*, and *S. hirsutum* which is freshly grown culture of fungal mycelium on MEA plates. Quantification was done according to Sambrook *et al.* (1982). So samples which gave the O.D. between 1.6-1.8 were used for Molecular identification of Timber Decay Fungi by RAPD (Plate I Fig. B).

Molecular identification of Timber Decay Fungi by RAPD

It is evident from the Table 1 that wood rotting fungi was collected from different areas of Gujarat was located. Initially 18 RAPD primers (20 primers Kit E, IDT USA; 160 primers, kit- J, K, L, N, O, P, Q, R, Operon technologies Inc., USA) were screened and out of which 15 primers responded with minimum 6 loci (bands) were included in the study. In the further screening 10 primers which gave fingerprints with good resolution and band reproducibility were used in the final analysis to characterize *L. sterioides, L. betulina, L. exima, F. flavus* strain 1, *F. flavus* strain 2, *G. lucidum, G. applanatum, P. robustus, S. commune, P. pulmonarius*, and *S. hirsutum*. In total 166 RAPD loci were generated and used to study molecular divergence and to deduce genetic relation among the genera.

RAPD analysis

Use of ten RAPD primers has produced totally 166 markers out of which 165 markers were found to be polymorphic. Markers obtained for each primer varied from 10 (OPL-5) to 22 (OPL-18) (Plate I Fig. C). On an average each primer produced 16.6 markers out of which 16.5 were polymorphic markers. Primer OPO-19 and OPN-6 has produced 1 common markers in all the species studied which is the highest number with any primer used in this study.

Table 1. Details of the geographic locations of collected fungal samples used in the present study

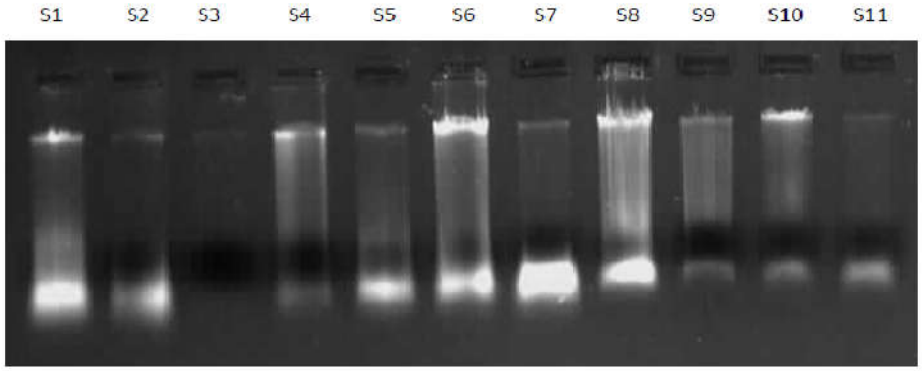
Serial number	Sample code	District provenance of collection (Gujarat)	Latitude	Longitude
1	L. S	Panchmahal	22° 46' 46" N	73° 39' 49" E.
2	Len A	Vadodara	25° 25' N	76° 70' E
3	Len a	Narmada	21.24° to 22° N	72.4° to 73.15° E
4	FFA	Vadodara	25° 25' N	76° 70' E
5	FF P	Panchmahal	22° 46' 46" N	73° 39' 49" E.
6	Gano p	Panchmahal	22° 46' 46" N	73° 39' 49" E.
7	Gano A	Vadodara	25° 25' N	76° 70' E
8	Phe	Narmada	21.24° to 22° N	72.4° to 73.15° E
9	Schizo	Vadodara	25° 25' N	76° 70' E
10	Pluer	Ahmedabad	23° 03' N	72° 40' E
11	Steri	Vadodara	25° 25' N	76° 70' E

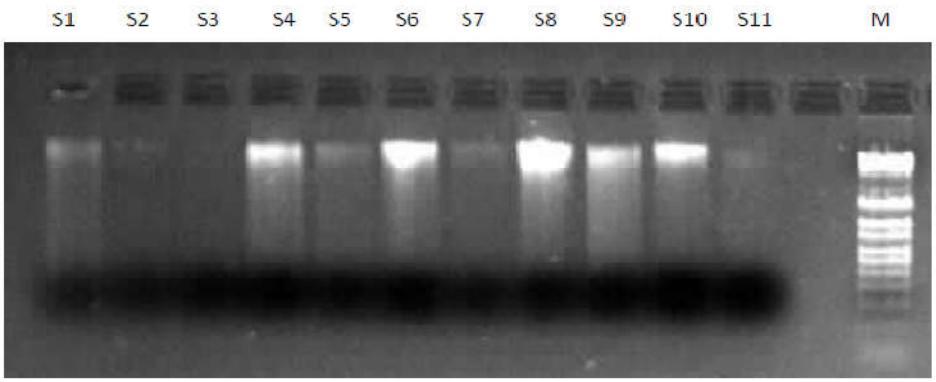
Table 2. RAPD primers showing the percentage of polymorphism/similarity in different timber rotting fungi

Primer Name	No. of makres	Percentage of polymophism	percentage of similarity
OPL-1	16	100	0
OPL-5	10	100	0
OPO-7	15	100	0
OPL-14	21	100	0
OPL-18	22	100	0
OPN-10	16	100	0
OPL-4	12	100	0
OPO-19	16	93.75	6.25
OPQ-15	18	100	0
OPN-6	20	95	5
	Mean	98.87	1.12

Table 3. RAPD Genetic similarity of different wood rotting fungi

	2	3	4	5	6	7	8	9	10	11
1	0.693									
2	0.651	0.596								
3	0.633	0.590	0.789							
4	0.530	0.560	0.578	0.584						
5	0.620	0.614	0.693	0.747	0.560					
6	0.620	0.627	0.729	0.675	0.572	0.627				
7	0.560	0.518	0.633	0.602	0.524	0.687	0.590			
8	0.584	0.554	0.645	0.699	0.548	0.639	0.651	0.566		
9	0.590	0.584	0.651	0.657	0.602	0.608	0.669	0.620	0.777	
10	0.560	0.578	0.620	0.627	0.548	0.639	0.602	0.566	0.614	0.548





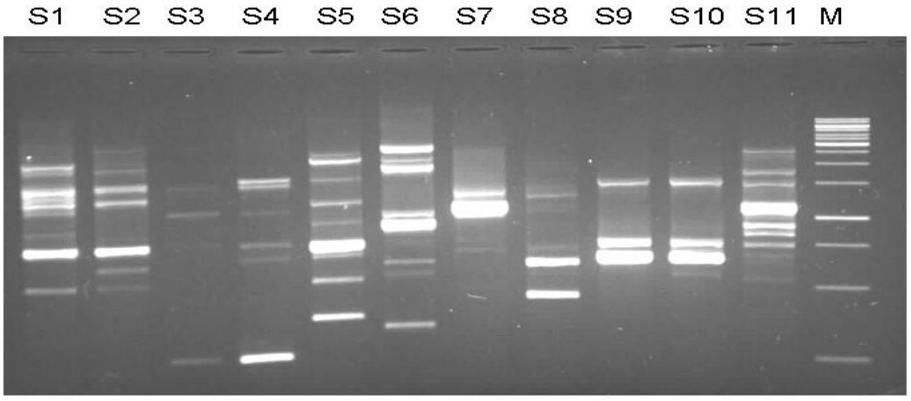


Plate I Fig A. Isolation of DNA from wood decay fungal 1 to11, B. genomic DNA Quantification from wood decay fungal 1 to11, C. RAPD banding patterns obtained by using OPO19 marker for wood decay fungal samples 1 to11

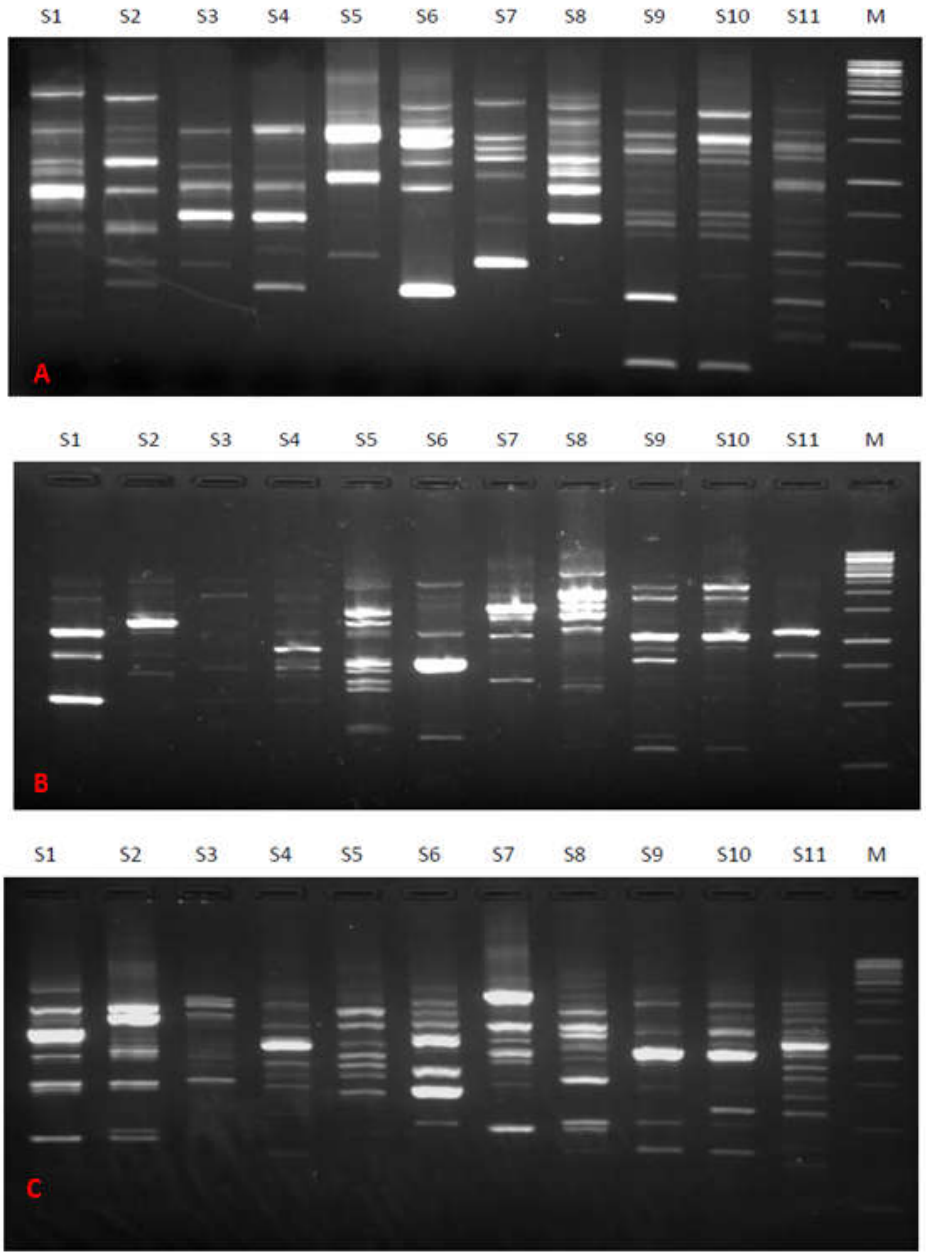


Plate II Fig A. RAPD banding patterns obtained by using OPN10 marker for wood decay fungal samples 1 to11, B. RAPD banding patterns obtained by using OPO7 marker for wood decay fungal samples 1 to11, C. RAPD banding patterns obtained by using OPL14 marker for wood decay fungal samples 1 to11

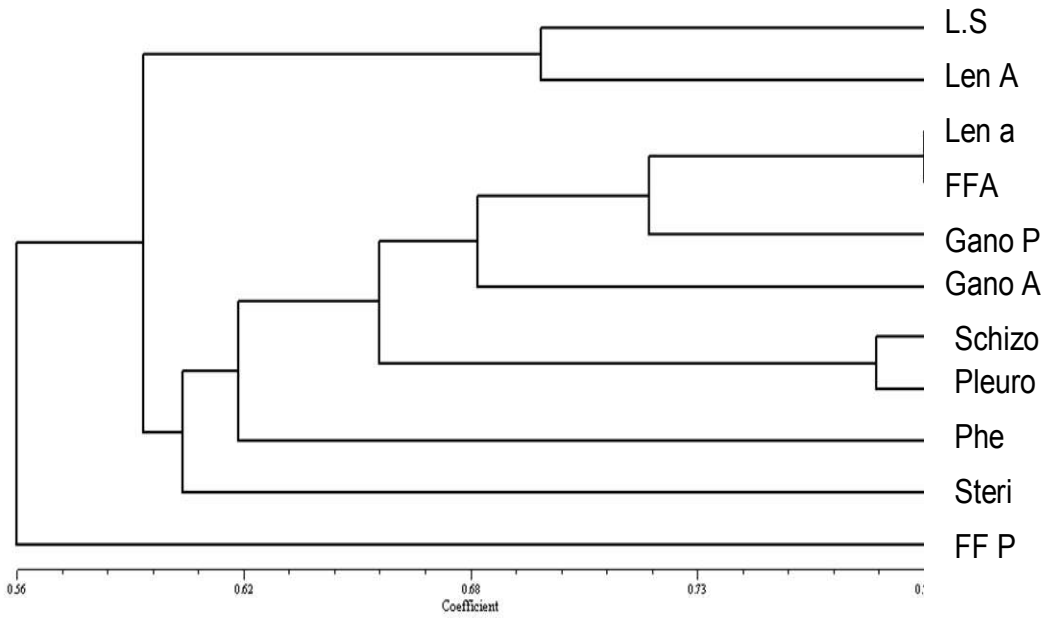
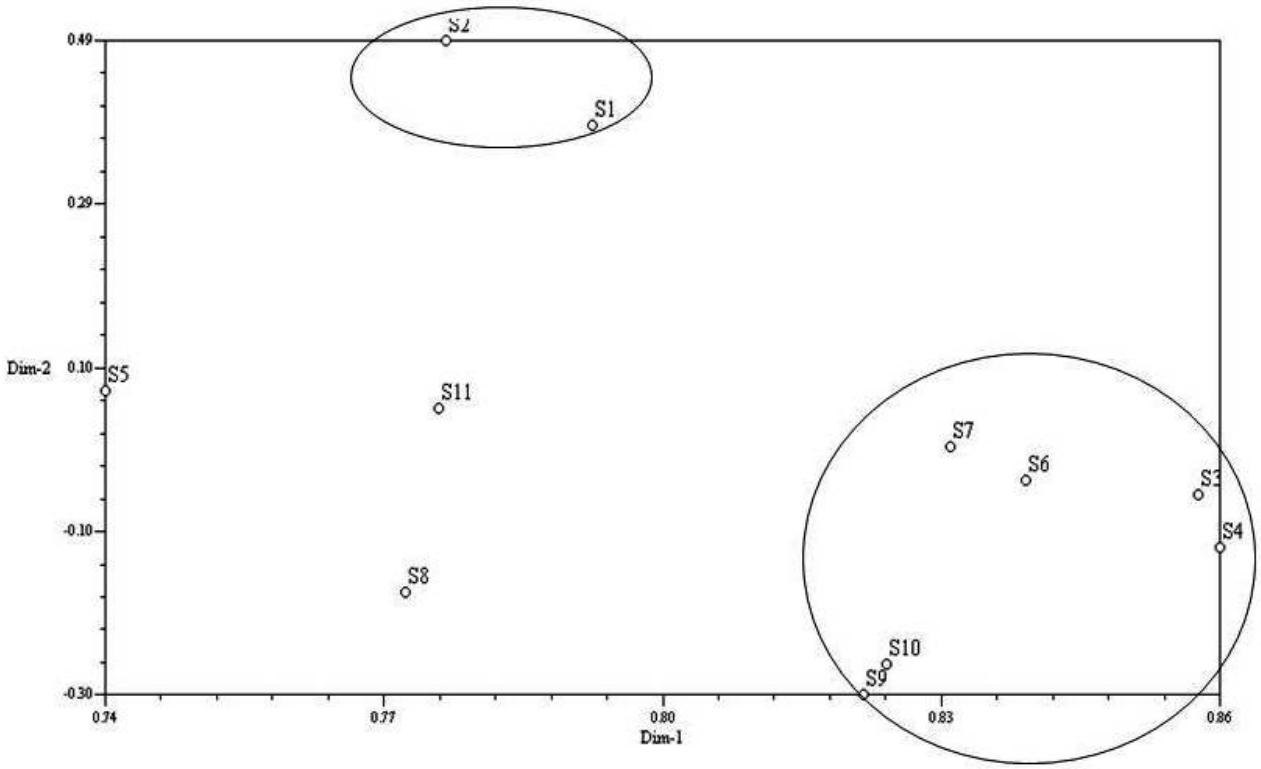


Plate III Fig A: Dendrogram of RAPD based on genetic similarity coefficient



Histogram 1: RAPD Percentage of cumulative analysis of different wood rotting fungi

100 percent polymorphism was observed when primers OPL-1, OPL-5, OPO-7, OPL-14, OPL-18, OPN-10, OPL-4, and OPQ-15. Use of primer OPL-5 has resulted in lowest number of markers (10) without any common marker and OPO19 has given lowest number of markers (16) with one marker common to all the species studied and showed lowest Percentage Polymorphism (PP) (93.75) (Plate II Fig A,B,C). The Genetic similarity (GS) between *L. sterioides* and *L. betulina* is 0.693, the genetic similarity between *L. betulina* and *L. exima* is 0.596, the genetic similarity between *L. exima* and *F. flavus* strain 1 is 0.789, the genetic similarity between *F. Flavus* strain 1 and *F. flavus* strain 2 is 0.584, the genetic

similarity between *F. flavus* strain 2 and *G. lucidum* is 0.56, the genetic similarity between *G. lucidum* and *G. applanatum* is 0.627, the genetic similarity between *G. applanatum* and *P. robustus* is 0.59, the genetic similarity between *P. robustus* and *S. commune* is 0.566, the genetic similarity between *S. commune* and *Pleurotus* sp. is 0.777, the genetic similarity between *P. pulmonarius*, and *S. hirsutum* is 0.548. On the contrary intrageneric GS was found maximum in between *L. exima* and *F. flavus* strain 1 and minimum in between *P. pulmonarius*, and *S. hirsutum*. Over all PP among seven genera through RAPD was found to be 98.81 with 2 markers common among all genuses studied. In the pair wise comparison the mean PP was 98.87 (Table 2 & 3, Histogram 1).

The dendrogram generated according to Jaccard [1908] from the binary data of RAPD. Based on the 166 RAPD fragments, dendrogram was prepared to determine the relationship between 7 genera of wood rotting fungi Aphyllophorales. The 7genera were clearly divided into two groups I and II. The II group includes the *F. flavus* strain 2 separated from all wood rotting fungi studied. The main group I is sub grouped into A and B. the sub group A includes the *L. sterioides* and *L. betulina* with approximately 60% similarity. The sub group B includes the *P. robustus* as sub group B1 with in the same sub group. The other wood rotting fungi form the sub group B2 with the same sub group of B. the *L. exima* and *F. flavus* strain 1 showed 100% similarity in RAPD dendrogram. The *Pleurotus* sp. and *S. commune* showed approximately 90% genetic similarity. The *G. Lucidum* showed around 70% similarity with *L. exima* and *F. flavus* strain 1. The *G. applanatum* is forming into a separate group whereas the *G. lucidum* is showing some similarity with *G. applanatum* forming as separate cluster (Plate III Fig A). The term DNA fingerprint too often is used improperly to refer to any complex pattern of DNA bands on a gel. Before a banding pattern can be classified correctly as a DNA fingerprint, a thorough statistical analysis is needed to validate its unique properties. The analysis generally is simple, based on using frequencies of individual bands within populations to calculate the probability that two randomly chosen individuals will share the same band pattern. DNA fingerprints based on both RFLPs and RAPDs have been widely used in fungi (Rosewich and McDonald, 1994). Their primary use is to identify clones or clonal lineages with a high degree of confidence. Their utility in population genetics can be extended beyond identification of clones by conducting genetic analysis to determine the molecular diversity and genetic relationships among the individual using amplicons in the DNA fingerprint. DNA fingerprints undoubtedly will be as useful for soilborne fungi and successfully used for identification characterization as they have been for other fungi (McDonald 1997). The large area to flourish is fungal plant pathology, because previous limits imposed by fastidious culture requirements (often limited to hosts) difficult genetics owing to a refractory sexual phase, the inability to obtain mutants and long life cycles have been bypassed with direct molecular study, including genomic analysis and intraspecific comparisons by RAPD technique (Valent and Chumley 1991, Kistler and Miao 1992). The five RAPD primes (CRL-1, 2,7,11,34) produced reproducible and consistent banding patterns. For each primer, the RAPD banding patterns generated could differentiate the *Ganoderma* isolates from different hosts (Zakaria *et al.,* 2009). In the present study also the RAPD primes produced reproducible and consistent banding patterns and also a single primer itself showed variation in the banding patterns in the wood rotting fungi under study to separate from each other. Within the same species small variations of banding patterns were observed as reported earlier (Zakaria *et al.,* 2009). In the present study also same results were observed when same genera were used for RAPD analysis. The Banding pattern of RAPD showed variations in some of the *G. boninese* isolates from oil plam (Zakaria *et al.,* 2009). In the present study the RAPD analysis differentiated the different genera into groups based on the variations in banding patterns.

To develop a method for the discrimination of basisiomycetes species and strains with vegetative mycelia, DNAs isolated from the mycelia of *Coprinus* and *Tricholoma* strains, were subjected to random amplified polymorphic DNA (RAPD) analysis. Seven *Coprinus* species could be distinguished, clearly showing species-specific DNA patterns in the RAPD analysis (Yasuhiro and Yanagi 1999). In the present study also the 3 species of *Lenzites* clearly showing species specific DNA pattern. One species of unknown *Coprinus* strain was identified as *C. cinereus* by this method. Six strains of *C. cinereus* and 4 of *C. angulatus* could also be distinguished by the presence of strain specific RAPD fragments. Five members of the *Tricholoma* family, *T. matsutake* and related 4 species, also showed species – specific DNA patterns in the RAPD analysis. The discrimination of *Tricholoma* species was confirmed by cluster analysis based on the 192 RAPD fragments (Yasuhiro and Yanagi 1999). In the present study the five member of polyporaceae family, 3 species of *Lenzites*, 2 species of *Ganoderma*, 2 strains of *F. Flavus*, also showed species specific DNA patterns in the RAPD analysis. This discrimination of polyporaceae members was confirmed by dendrogram generated based on the genetic similarity of 166 RAPD fragments. The 5 species could be clearly divided into 5 groups in complete agreement with the taxonomic classification. RAPD analysis of mycelial DNA is a suitable method for distinguishing basidiomycetes species and strains (Yasuhiro and Yanagi 1999). Very few studies were made towards this aspect using the AFLP and in the present study we could get very consistent results and most of the observations made following result of RAPD. The present part of work, Molecular characters of certain Timber Degrading Fungi using RAPD is the novel study where the results were compared and contrasted among the two most used and reported to be very competitive techniques. As reported earlier in higher plants kingdom, the present study conducted concludes that RAPD techniques gave good consistent conclusions. The present study will pave way further exploitation of the molecular marker techniques for identification, characterization, diversity analysis, to deduce the phylogenetic relations among the new group of fungi and exploitation of these markers for the improvement of industrially important fungal strains.

Conclusion

They were characterized molecularly with markers. Analyses of genetic relationship of different timber degrading Aphyllophorales germplasm were conducted using RAPD. Based on RAPD analysis 100 percent polymorphism was observed when primers OPL-1, OPL-5, OPO-7, OPL-14, OPL-18, OPN-10, OPL-4, and OPQ-15. Use of primer OPL-5 has resulted in lowest number of markers (10) without any common marker and OPO19 has given lowest number of markers (16) with one marker common to all the species studied and showed lowest Percentage Polymorphism (PP) (93.75). On the contrary intrageneric GS was found to be

maximum in between *L. exima* and *F. flavus* strain 1 and minimum in between *P. pulmonarius*, and *S. hirsutum*. Over all PP among seven genera through RAPD was found to be 98.81 with 2 markers common in the entire all genuses studied. In the pair wise comparison the mean PP was 98.87.

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