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MOLECULAR CHARACTERIZATION OF WILD EDIBLE MUSHROOMS FROM MADHYA PRADESH (INDIA) USING RAPD MARKERS

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ABSTRACT
Seventeen wild edible species of mushrooms were collected from five major tribal districts of Madhya Pradesh, India. Thirty RAPD primers were used to characterize these seventeen species in order to analyze their genetic diversity. The RAPD primers used were able to amplify 304 loci in the genomes. Though all species could be characterized using RAPDs, no single primer could differentiate all of them. The greatest difference observed between two isolates was, with similarity of only 3.1% while the minimum difference was observed with similarity of 35.3%. The RAPDs revealed sufficient polymorphism to discriminate different species collected from various regions of Madhya Pradesh viz., Jabalpur, Mandla, Balaghat, Dindori and Shahdol.

INTRODUCTION

Mushrooms have been the major source of proteins, minerals and vitamins for humans since time immemorial. They supplement and complement the nutritional deficiency in cereals and are the highest producers of protein. They are recognized as natural and healthy foods originating from an environment friendly ecosystem (Moore *et al*., 2001). Therefore, to make mushrooms more common among people nutritionally and palatably, novel strains with greater nutritional and economic value should be explored. Mushrooms have great potential in food, pharmaceuticals and cosmetic industry. Central India has a wide diversity of edible mushrooms both at generic and species level. Major districts of Madhya Pradesh (central India) with mushroom production and consumption, lies in tribal areas of the state, having mushrooms as their major source of protein. However, wild but edible, mushrooms of this region have still not received any attention regarding their genetic and molecular make-up.

Mushrooms belong to a group of basidomycete fungi. Being morphologically diverse, strains are difficult to discriminate due to lack of clearly distinguishable characters (Pei-Sheng *et al*., 2004).

There are a number of problems in classifying mushrooms by only using morphological characters which are often unreliable and inconclusive due to large influence exerted by environmental factors or compatibility experiments (which are based on the application of the controversial biological species concept) (Zervakis *et al*., 2001). Therefore characterization based on molecular markers could open new avenue for their identification and cultivation. The genetic marker screening is based on the survey of genetic diversity as revealed by variation at specific gene loci and provides information about the amount and distribution of genetic diversity within and among populations (Buu and Lang, 1999). Among these, the most convenient and easy marker system with low input and effort are the RAPDs. It does not require any information about the DNA sequence to be amplified (Weder, 2002) and hence, can be used for scanning a number of unknown genomes. The use of Random Amplified Polymorphic DNA (RAPD) (Williams *et al*., 1990) offers an additional advantage of generating a large number of markers for genome mapping without prior knowledge of gene sequence. The RAPD technique offers a powerful tool to discriminate species and provide useful tool for their breeding (Chandra *et al*., 2010). Hence, the present study was undertaken to characterize relatively unknown but important mushroom species that are edible but grow wild in Madhya Pradesh.

MATERIALS AND METHODS

Seventeen species of mushroom (Table 1) were collected through extensive field visits from the forest region of five major districts (Dindori, Mandla, Shahdol, Balaghat, and Jabalpur) of Madhya Pradesh, India with the help of local

tribal who were aware about the edibility of these wild mushrooms, and frequently used them as a part of their regular diet. The samples from the forests were carefully secured in insulated boxes with ice packs, in order to avoid any deterioration caused in long journey to the lab.

Morphological identification of wild species

Morphological identification of the collected samples was done at the Tropical Forest Research Institute, Jabalpur.

Genomic DNA isolation

The stalk of the fruiting bodies was used for extracting DNA. The outer cover was removed with a scalpel and the inner soft tissue was used for DNA extraction. Zymo Research Kit (Biomatrix) for fungal DNA isolation was used to extract DNA from stalk.

RAPD-PCR and data analysis

Amplification reaction for RAPD-PCR was performed using thirty decamer primers obtained from Operon Technologies. The reaction was performed in 20 µl reaction volume containing 1X buffer, 2.5 mM $MgCl_2$, 250 µM each of dNTPs, 1 U of Taq polymerase and 50 ng primers. PCR was performed with 45 cycles of **:** 45 s at 94ºC, 1 min at 36ºC, and 2 min at 72ºC, and a final extension of 7 min at 72ºC. The PCR products were separated by electrophoresis on 1.2% agarose gels. One kb DNA ladder was used as a molecular size marker. The gel was run for 3 hours at 50 V. The fragments were visualized by staining with ethidium bromide and photographed on gel documentation system. Genetic relationship among the isolates was established using similarity coefficients calculated between isolates and a dendrogram was drawn using Unweighted Pair Group Method using arithmetic averages algorithm (UPGMA).

RESULTS AND DISCUSSION

The present work is the first of its kind for the molecular characterization of wild and extensively edible species of mushrooms in Madhya Pradesh, India. Phylogenetic analysis of the collected samples using RAPD revealed large interspecific and intergeneric genetic variability, indicating, that RAPD can be applied to determine the extent of diversity in mushroom breeding materials intended for genetic crossings (Khush *et al.*, 1991; Khush *et al.*, 1992). RAPD markers are superior when simplicity and costs are considered (Williams *et al.*, 1990). RAPD patterns are consistent irrespective of the source or age (Micheli *et al.*, 1994). The use of RAPDs have been known in different well known species and varieties in mushroom for their genetic discrimination due to their ease of working (Staniaszek *et al.*, 2002; Fonseca *et al.*, 2008) and

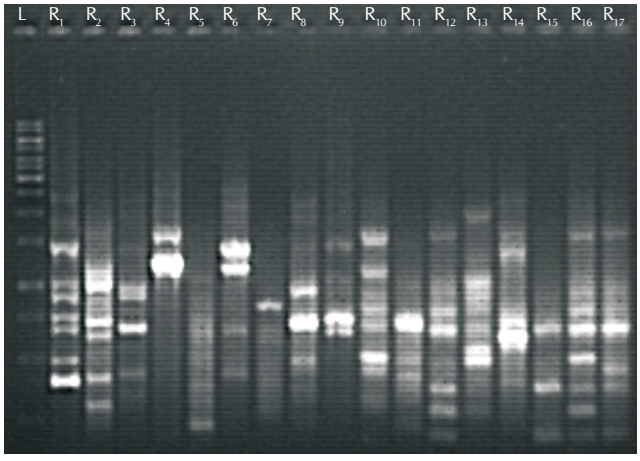

Figure1: The RAPD banding profile of primer OPA 09

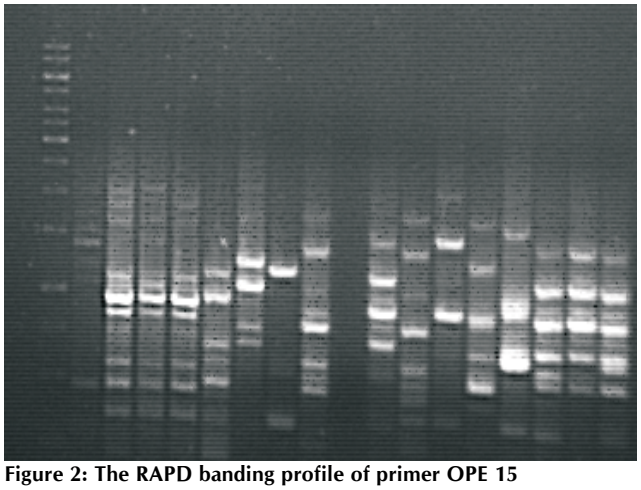

Figure 2: The RAPD banding profile of primer OPE 15

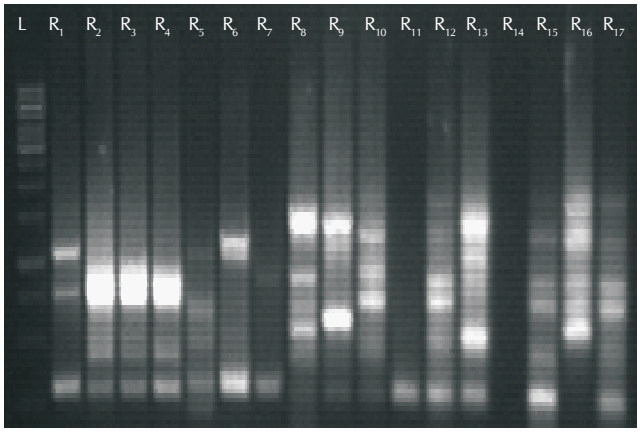

Figure 3: The RAPD banding profile of primer OPG 02

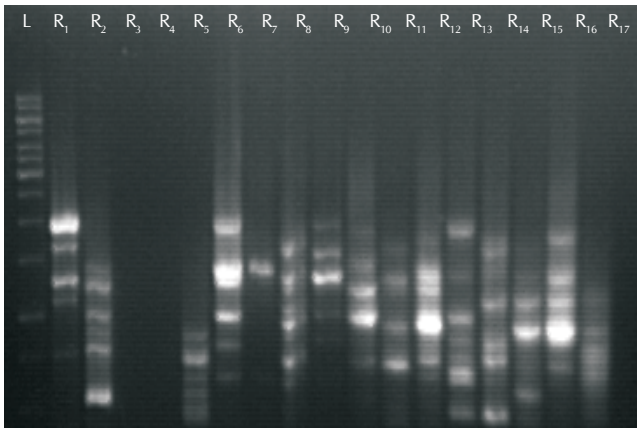

Figure 4: The RAPD banding profile of primer OPL 11

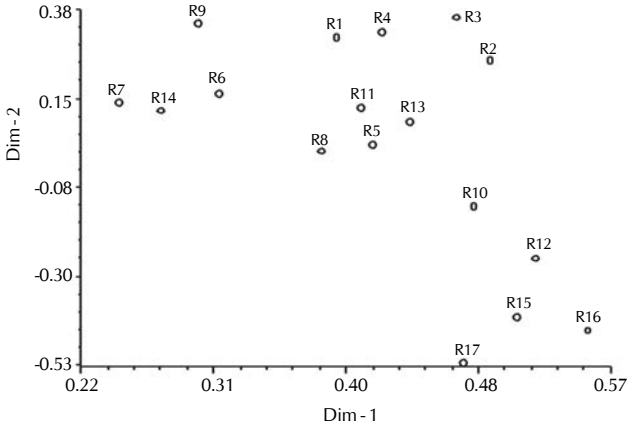

Figure 5: Cluster diagram based on twenty eight RAPD primers of seventeen mushroom species

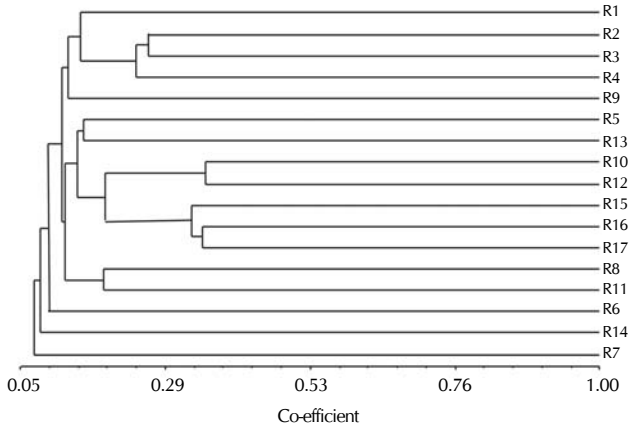

Figure 6: Dendrogram based on twenty eight RAPD primers in seventeen mushroom species

Table 1: Species collected and identified on the basis of mycological characteristics (serial number corresponds to lane number)

Species number	Species	Family	Place of collection
R_1	*Astraeus hygrometricus*	Astraeaceae	Dindori
R2	*Lepiota procera*	Lepiotaceae	Jabalpur
R_3	*Ganoderma lucidum*	Ganodermataceae	Jabalpur
R_4	*Ganoderma spp.*	Ganodermataceae	Jabalpur
R_5	*Russula spp.*	Russulaceae	Dindori
R_6	*Agaricus campestris*	Agaricaceae	Dindori
R_7	Unidentified	-	Jabalpur
R_8	Unidentified	-	Balaghat
R_9	*Scleroderma spp.*	Astraeaceae	Shahdol
R10	*Termitomyces hemi*	Lyophyllaceae	Shahdol
R11	*Termitomyces spp.*	Lyophyllaceae	Balaghat
R12	*Termitomyces hemii* Natarajan	Lyophyllaceae	Balaghat
R13	*Cantherellus spp.*	Cantharellaceae	Jabalpur
R14	*Calocybe spp.*	Lyophyllaceae	Jabalpur
R15	*Termitomyces hemii* Natarajan	Lyophyllaceae	Mandla
R16	*Termitomyces spp.*	Lyophyllaceae	Jabalpur
R17	*Termitomyces hemii* Natarajan	Lyophyllaceae	Balaghat

Table 2: RAPD primers used with the respective number of amplified bands

S.No.	Primers	Sequence 5′- 3′	No. of bands amplified
1.	OPN 04	GACCGACCCA	42
2.	OPN 15	CAGCGACTGT	0
3.	OPO 03	CTGTTGCTAC	8
4.	OPG 11	TGCCCGTCGT	17
5.	OPA 09	GGGTAACGCC	36
6.	OPF 14	TGCTGCAGGT	0
7.	OPH 19	CTGACCAGCC	24
8.	OPA 04	AATCGGGCTG	24
9.	OPA 01	CAGGCCCTTC	14
10.	OPH 17	CACTCTCCTC	15
11.	OPL 06	GAGGGAAGAG	3
12.	OPS 19	GAGTCAGCAG	42
13.	OPS 14	AAAGGGGTCC	11
14.	OPA 07	GAAACGGGTG	9
15.	OPL 01	GGCATGACCT	23
16.	OPP 07	GTCCATGCCA	26
17.	OPA 02	TGCCGAGCTG	32
18.	OPP 19	GGGAAGGACA	61
19.	OPS 03	CAGAGGTCCC	33
20.	OPG 02	GGCACTGAGG	98

providing rapid results in short time and less expense (Arif and Khan, 2009; Temiesak *et al*., 1993).The use of RAPD not only reveals higher polymorphism between different genera (15), it also produces substantial polymorphism within species to differentiate them. RAPD primers can distinguish taxa below the species level (Choo *et al*., 2009), because RAPD analysis reflects both coding and non-coding regions of the genome (Vanijajiva *et al*., 2005).

The RAPD banding profile of highly polymorphic primers is illustrated in Figs. 1-4. Thirty RAPD primers were used to amplify seventeen wild species of mushroom. Out of thirty primers screened, two primers were unable to amplify any species or genus. The PCR reaction of the seventeen species using twenty-eight RAPD primers (Table 2) produced 833 bands ranging between 250 bp to 2500 bp with a number of bands for each primer ranging between 3 and 98. The most informative and scorable RAPD primers with respect to the number of amplified fragments and total polymorphism were OPA 09 (Fig. 1), OPE 15 (Fig. 2), OPG 02 (Fig. 3), OPL 11 (Fig .4). For the data analysis, each band was regarded as a locus with two alternative alleles: present (1) or absent (0) (Lynch and Milligan, 1994). The gel image data was used to generate a dendrogram (Fig. 6). The dendrogram was generated by UPGMA analysis and clustering indicated grouping of these mushrooms (Fig .5). The similarity coefficient ranged between 0.031-0.353. The highest similarity coefficient was obtained between the two *Termitomyces* species of 0.353, while the lowest was obtained between the other two *Termitomyces* species of 0.031. The inconspicuously visible bands were excluded from scoring.

The number of primers used in the study was found to be sufficient to characterize all the seventeen different species. Though many primers produced less number of bands, they still revealed enough polymorphism to differentiate these species. These primers amplified 304 loci, clearly scorable and were used for generating dendrogram. The maximum number of clear bands was produced by primer OPP 12, while the minimum bands were produced by primer OPS 06.

Cluster analysis

The cluster analysis clearly illustrated the presence of three major clusters with many sub-clusters in it indicating possible similar phylogeny of the clustered species. According to the molecular markers used there were three broad clusters formed comprising CLU 1 with species R6, R7, R9 and R14, CLU 2 with R1, R2, R3, R4, R5, R8, R11, R13 and CLU 3 with R10, R12, R15, R16, R17. The analysis showed that there were very few common loci amplified for all the species with respect to one primer. For instance, primer OPN 04 produced 42 bands; and no single band was evenly present in all species. Therefore, this level of polymorphism generated by RAPDs was very informative regarding their phylogenetic study. The species under study were difficult to be distinguished based on morphological characters.

RAPD markers as important molecular tool

The RAPD markers have proved to be an essential tool to scan the unknown genome of any organism. However, complete dependence on these markers for virtual characterization is still not reliable due to their random nature. One should be very careful in interpreting the data generated by these markers. In spite of these shortcomings these markers have gained worldwide acceptance due to their ease of work and quick, simple results. In short time span, they not only amplify a number of loci in the genome but also exhibit wide polymorphism even among closely related species. Hence, these markers provide initial working knowledge of the unknown genome to start with.

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