International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **2(10),** 1-5, October **(2013)** *Int. Res. J. Biological Sci.*

Genomic Discrimination of Eleven Commercial Mushrooms by DNA Fingerprinting using RAPD Marker

Agarwal K., Prasad M.P.* and Rindhe G.
Sangenomics Research Labs, 16, 2nd Cross, Krishna Reddy Colony, Domlur Layout, Airport Road, Bangalore- 560071, INDIA

Available online at: www.isca.in, www.isca.me
Received 23rd May 2013, revised 5th June 2013, accepted 21st July 2013

Abstract

In today's world with burgeoning population the growth cultivation of edible mushrooms holds great significance because of their nutritive and therapeutic properties. The advances in research on mushroom breeding and production are highly constrained compared to other crops; due to lack of previous knowledge in mushroom genetic. Present study focuses on establishing phylogenetic relationship among the eleven elite edible mushrooms using RAPD markers. Eleven mushroom samples namely 1) A. bisporus (Button), 2) A. bisporus (Portobello), 3) P. eryngii (King Oyster), 4) L. edodes (shiitake), 5) H. tessellatus (Brown Shimeji), 6) H. tessellatus (White Shimeji), 7) F. velutipes (Enoki), 8) P. ostreatus (Oyster), 9) P. djamor (Pink Oyster), 10) C. indica (Milky), and 11) P. florida (Florida Oyster) were used. Molecular markers serve as quick and reliable tool to establish the identities of mushrooms and are helpful in mushroom taxonomy. Among the various primers used to OPZ 10 gave the most distinguished and scorable band pattern. To estimate the similarity and genetic distance among different mushroom, cluster analysis based on frequency similarity was performed. The RAPD analysis in this study has proven to be useful in discrimination, characterization and differentiation of the fungal cultivars.

Keywords: Genetic diversity, DNA Fingerprint, Mushroom, RAPD.

Introduction

Since ancient time mushrooms have been cultivated and used as food, due to their nutritive and medicinal values especially in the eastern countries and recognized as natural and healthy foods[1]. The protein found in mushrooms is less than in animals but more than in most plants. They have low fat content, high fiber and all essential amino acids and with the exception of iron, contain all important minerals[2]. As time progress there was increase in awareness about mushrooms nutritive and medicinal value[3]. Use of medicinal mushrooms in Western countries has increased during the last decade but in Asian countries, it has long tradition[4]. Mushrooms are rich sources of nutraceuticals[5,6] and their bioactive properties have been proven by the scientific community[7]. Also, it is very environmental friendly, capable of converting the lignocellulosic waste materials into food, feed and fertilizers[8,9]. Mushrooms and their metabolites are used as adaptogens, immunostimulants and antitumor agent and for curing many of human diseases[10]. Reports show that mushrooms show antitumor, antibacterial, antiviral and haematological activity and used in immunomodulating treatments[11] also possess antioxidant capacity[12] and can be used as food supplement as well as in the pharmaceutical industry.

Molecular markers such as rDNA sequencing, RFLP, RAPD and genotyping have been used to discriminate mushroom species or strains of *Agaricus*, *Auricularia*, *Ganoderma, Lentinula*, *Stropharia, rugoso-annulata* and *Volvariella*. All of these technologies provided data for mushroom strain identification and protection[13]. RAPD analysis was first developed to detect polymorphism between organisms, despite the absence of sequence information, to produce genetic markers, and to construct genetic maps[14]. Genetic diversity of mushrooms has been determined previously using molecular markers especially RAPD[15,16] and this technique is used to assess the genetic diversity among 37 pleurotus species of mushrooms and found that, this technique provide better discrimination than morphological analysis[17]. But due to lack of clearly distinguishable characters, mushroom samples are very difficult to discriminate and hence create problems in sample protection and improvement[18]. In this study the discrimination has been done by RAPD technique to achieve the genetic diversity among them.

Material and Methods

Sample Collection: A total of eleven samples of mushrooms viz. *1) A. bisporus (Button), 2) A. bisporus (Portobello), 3) P. eryngii (King Oyster), 4) L. edodes (shiitake), 5) H. tessellatus (Brown Shimeji), 6) H. tessellatus (White Shimeji), 7) F. velutipes (Enoki), 8) P. ostreatus (Oyster), 9) P. djamor (Pink Oyster), 10) C. indica (Milky), and 11) P. florida (Florida Oyster)* were collected from local market in Bangalore. The samples were identified at Indian Institute of Horticultural Research (IIHR) Hessaraghatta, Bangalore.

DNA Extraction: DNA extraction was performed as reported[19] with following modifications. Approximately 50 mg of fresh mushroom samples were cut into small pieces and grinded with homogenization buffer (50 mM Tris, 10 mM EDTA and 50 mM

glucose) and extraction buffer (100 mM Tris, 10 mM EDTA, 250 mM NaCl and 1% Sodium Dodecyl Sulfate, pH= 8.0) with Proteinase-K and incubated in dry bath at 60^0C for 1 h. After centrifugation at 3,000 rpm for 5 min., supernatant was treated with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1), incubated at 37^0C for 10 min and centrifuged at 8,000 rpm for 6 min. The Phenol: Chloroform step was repeated and supernatant was collected. The DNA was precipitated using equal volumes of chilled isopropanol. The DNA samples were tested qualitatively on 0.8% agarose gel and quantified by Nanodrop ND 1000 spectrophotometer (Thermo Scientific).

PCR Reaction: The PCR was carried out in final volume of 25 µl containing 100 ng DNA, 2 U of Taq DNA polymerase, 2.5 mM $MgCl_2$, 1.5 µl of 2.5 mM each dNTPs (Chromous Biotech, Bangalore) and 100 pmol of primers (Eurofins Genomics, Bangalore). The DNA amplification was performed in the Corbett RG 6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94°C for 45 sec, 32°C for 45 sec and 72°C for 45 sec) followed by 30 cycles of amplification (94°C for 30 sec, 34°C for 30 sec and 72°C for 30 sec) and the final elongation step (72°C for 5 min).

Gel electrophoresis and Data Analysis: Total volume of the amplified product (25µl) of each sample was subjected to electrophoresis on 1.5 % agarose gel containing ethidium bromide in 1x TAE buffer at 90 V for 1 h. Finally, the DNA bands were observed on a Gel Doc system and the photographs were captured. The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the frequency similarity. The matrix of genetic distance was used for grouping the mushroom samples based on the dendrogram constructed by UPGMA (Unweighed Pair Group Method with Arithmetic averages).

Results and Discussion

The genomic DNA from the mushroom samples was isolated by Phenol-chloroform extraction method. The qualitative estimation of the DNA on 0.8% agarose gel gave single, sharp and distinct bands devoid of any smear. Thus, genomic DNA of good quality without any degradation was successfully isolated from all the eleven samples. Here, the quantitative estimation of genomic DNA was done by Thermo Scientific Nanodrop 1000 spectrophotometer. The genomic DNA were obtained in high concentration for all the samples and they showed a good 260/280 ratio (i.e. between 1.8 and 2.0) indicating absence of any protein or RNA contaminants.

Ten random primers were used for RAPD DNA fingerprinting table 1. Out of ten primers used, four primers namely OPS 5, OPT 5, OPW 2, and OPZ 10 produced clear banding patterns. Among these primers OPZ 10 produced the most distinguished and scorable band pattern (followed by OPT 5). OPS 5 produced a total 45 bands ranging from 1-8, OPT 5 produced a total 85 bands ranging from 4-11, OPW 2 produced a total 77 bands ranging from 2-10 for the eleven samples. OPZ 10 produced a total 96 bands ranging from 4-16 for the eleven samples figure 1.

Table-1
The sequence and annealing temperatures of primers used

No.	Oligo Name	Sequence(5'→3')	Tm (^{o}C)
1.	OPA 8	GTGACGTAGG (10)	32.0
2.	OPD 20	ACCCGGTCAC (10)	34.0
3	OPR 9	TGAGCACGAG (10)	32.0
4.	OPS 5	TTTGGGGCCT (10)	32.0
5.	OPT 5	GGGTTTGGCA (10)	32.0
6.	OPU 10	ACCTCGGCAC (10)	34.0
7.	OPV 1	TGACGCATGG (10)	34.0
8.	OPW 2	ACCCCGCCAA (10)	34.0
9.	OPX 6	ACGCCAGAGG (10)	34.0
10.	OPZ 10	CCGACAAACC (10)	32.0

Different number of bands was observed in PCR for different primers which might be due to the sequence of primer and availability of complementary sequence in the genome or template quality. All bands showed polymorphism and there was no monomorphic band observed common to all the samples.

The DNA fingerprint generated by each RAPD primer was scored for presence or absence of specific bands. In order to estimate the genetics distance among different mushroom, cluster analysis was performed based on frequency similarity with weighted pair-group with arithmetic average (UPGMA) by Alpha Imager HP and dendrogram was constructed. The lowest frequency similarity coefficient for OPS 5, OPT 5, OPW 2 and OPZ 10 were found to be 0.09, 0.07, 0.09 and 0.18 respectively. Whereas, the highest frequency similarity coefficient for were found to be 0.34, 0.20, 0.29 and 0.38 respectively.

Since, OPZ 10 gave the best banding pattern it used for interpretation of the phylogenetic relationship. Two main clusters were observed in the dendrogram. Grouping of samples in clusters exposed that, the morphological behavior of these samples was similar or they share the same ancestors. First cluster consisted of sample 2, 4 and 6 and second cluster consisted of sample 1, 3, 5, 7, 9, 10 and 11. Sample 8 lied as a separate entity. Sample 9 and 11 showed 100 % similarity and Cluster of 9 and 11 showed similarity with sample 3, 5, 7,10 and 1 in decreasing percentage (93.75, 87.5, 75, 75, 75) respectively. Sample 8 showed least similarity with all the other samples having a maximum match of (43.75 %) to samples 2 and 6 (first cluster).

From these results we can conclude that sample 9 and 11 might share same morphological or physiological characteristics. Sometimes, morphological grouping differ compared to molecular/genomic relationship among the species[18]. Therefore, RAPD technique can be used to interpret overall genetic relatedness and dissimilarity[15].

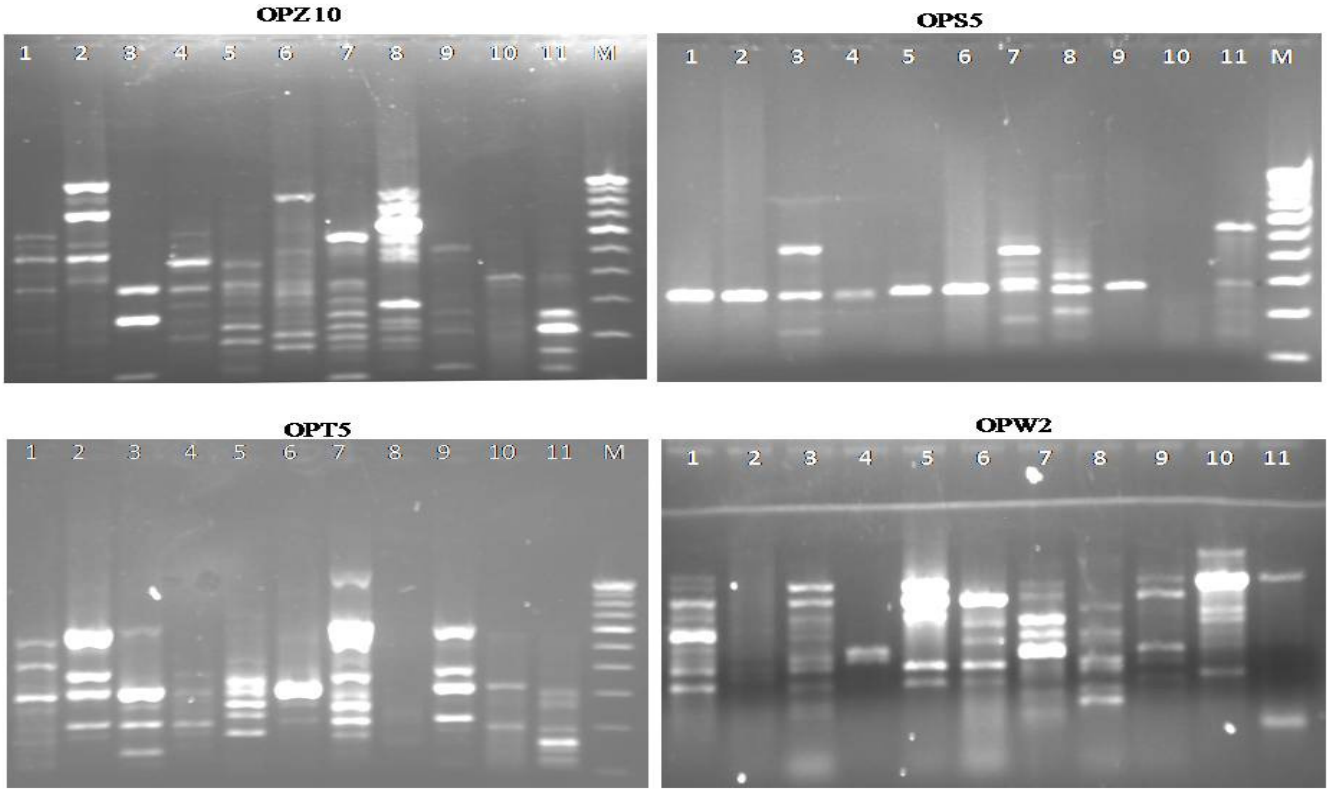

Figure-1
DNA fingerprinting of mushroom samples by primers OPZ-10, OPS-5, OPT-5 and OPW-2

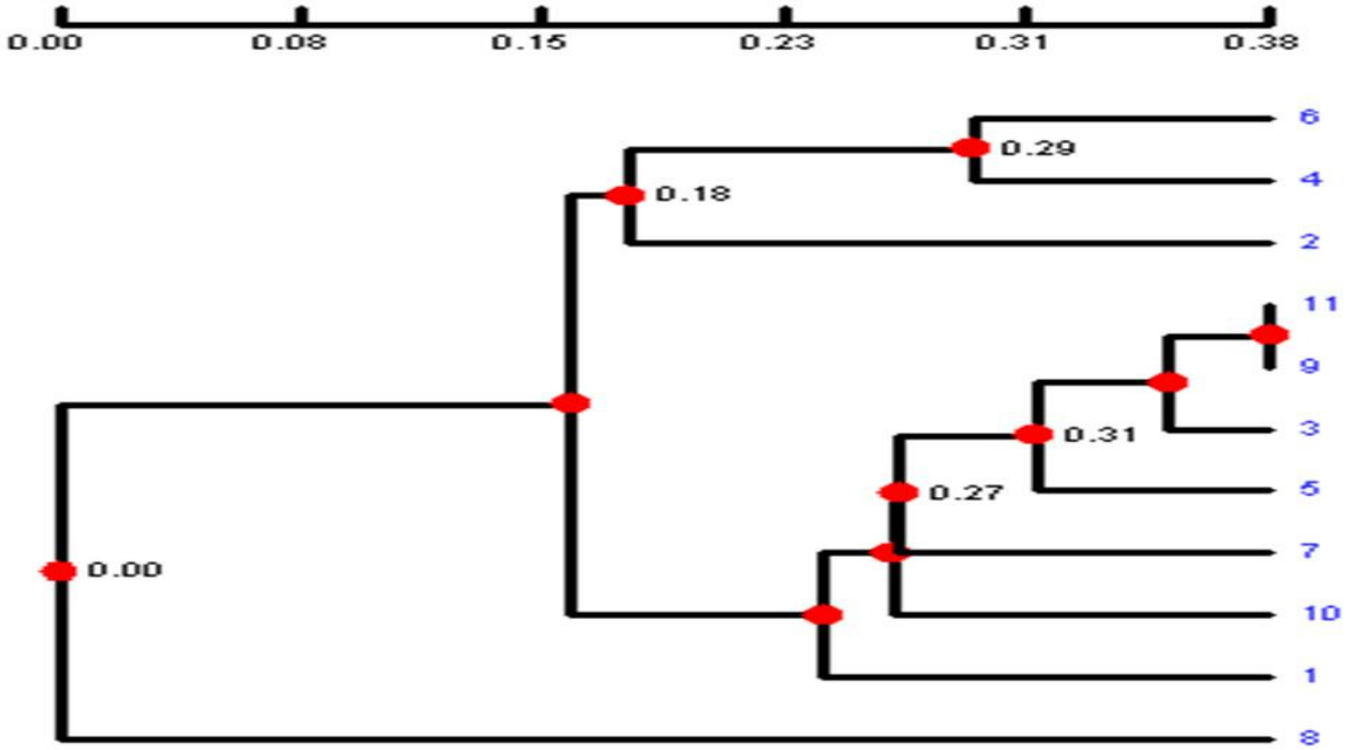

Figure-2
Dendrogram for amplified DNA for primer OPZ 10

Table-2
Frequency Similarity matrix for amplified DNA for primer OPZ 10

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	100	-	-	-	-	-	-	-	-	-	-
2	43.75	100	-	-	-	-	-	-	-	-	-
3	81.25	50	100	-	-	-	-	-	-	-	-
4	62.5	56.25	68.75	100	-	-	-	-	-	-	-
5	62.5	68.75	81.25	62.5	100	-	-	-	-	-	-
6	56.25	62.5	50	81.25	43.75	100	-	-	-	-	-
7	75	43.75	81.25	50	75	43.75	100	-	-	-	-
8	25	43.75	6.25	25	25	43.75	25	100	-	-	-
9	75	56.25	93.75	62.5	87.5	43.75	75	12.5	100	-	-
10	62.5	68.75	81.25	75	75	56.25	75	12.5	75	100	-
11	75	56.25	93.75	62.5	87.5	43.75	75	12.5	100	75	100

Conclusion

The RAPD analysis in this study has proven to be useful in discrimination, characterization and differentiation of the fungal cultivars and grouping them according to similarity. The banding pattern by RAPD was variable depending upon the primer. Genetic mapping of the mushroom genome will help in understanding their complex traits such as yield, size, colour, flavour and shelf-life. Thus, the exploitation of genetic resources to broaden genetic variability is promising to develop new cultivars with higher yield, enhanced flavor resistance to diseases or adaptation to climate changes with a greater shelf life.

References

1. Iwalokun B.A., Usen U.A., Otunba A.A. and Olukoya D.K., Comparative phytochemical evaluation, antimicrobial and antioxidant properties of Pleurotus ostreatus, *AJB*, **6 (15)**, 1732-1739 **(2007)**

2. Sadler M., Nutritional properties of edible fungi, *Br. Nutr. Found. Nutr. Bull*., **28**, 305-308 **(2003)**

3. Khan S.M., Nawaz A., Malik W., Javed N., Yasmin T., Rehman M.U., Qayyum A., Iqbal Q., Ahmad T. and Khan A.A., Morphological and molecular characterization of Oyster mushroom (Pleurotus spp.), *AJB*, **10(14)**, 2638-2643 **(2011)**

4. Sharma N., Medicinal uses of macrofungi, *Ethnobotan*, **15**, 97-99 **(2003)**

5. Çaglarirmak N., The nutrients of exotic mushrooms (Lentinulaedodes and Pleurotusspecies) and an estimated approach to the volatile compounds, *Food Chem*., **(105)**, 1188–1194 **(2007)**

6. Elmastas M., Isildak O., Turkekul I. and Temur N., Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms, *J. Food Comp. Anal*., **20**, 337–345 **(2007)**

7. Lindequist U., Niedermeyer T.H.J. and Julich W.D., The pharmacological potential of mushrooms, *eCAM*, **2(3),** 285-299 **(2005)**

8. Hadar Y., Keren Z., Gorodecki B., and Ardon O., Utilization of lignocellulosic waste by the edible mushroom Pleurotus. *Biodegradation*, **3**,189-205 **(1992)**

9. Jaradat A.A., Genetic resources of energy crops: biological systems to combat climate change, *AJCS*, **4**, 309-323 **(2010)**

10. Jose N. and Janardhanan K.K. Antioxidant and antitumor activity of Pleurotus florida, *Curr Sci*, **79(7)**, 941-943 **(2000)**

11. Yang J.H., Lin H.C. and Mau J.L. Antioxidant properties of several commercial mushrooms, *Food Chem.*, **77,** 229-235 **(2002)**

12. Ribeiro B., Rangel J., Valenta'o P., Baptista P., Seabra R. M. and Andrade P. B., Contents of carboxylic acids and two phenolics and antioxidant activity of dried portuguese wild edible mushrooms, *J. Agric. Food Chem.*, **54**, 8530–8537 **(2006)**

13. Chandra S., Ghosh K. and Acharya K. Comparative studies on the Indian cultivated Pleurotus species by RAPD fingerprinting. *Nature and Science*, **8(7)**, 90-94 **(2010)**

14. Williams J.G., Kubelik A.R., Livak K.J., RafalskiJ.A. and Tingey S.V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., **18 (22),** 6531-6535 **(1990)**

15. Ravash, R., Shiran, B., Alavi, A. and Zarvagis, J. Evaluation of genetic diversity in Oyster mushroom (Pleurotus eryngii) isolates using RAPD marker, *J. Sci. & Technol. Agric. & Natur. Resour*., **13**, 739-741 **(2009)**

16. Staniaszek M., Marczewski W., Szudyga K., Maszkiewicz J., Czaplicki A. and Qian G. Genetic relationship between Polish and Chinese samples of the mushroom Agaricus

bisporus (Lange) Sing., determined by the RAPD method, *J. Appl. Genet*., **43**, 43-47 (**2002)**

17. Stajic M., Sikorski J., Wasser S.P. and Nevo E., Genetic similarity and taxonomic relationships within the genus Pleurotus (higher Basidiomycetes) determined by RAPD analysis, *Mycotoxon*, **93**, 247-255 **(2005)**

18. Pei-Sheng Y., Luo X.C. and Zhou Q., RFLP analysis of amplified nuclear ribosomal DNA in the genus Auricularia, *Myco system*, **18,** 206–213 **(1999)**

19. Magdum S.S., A Reliable and High Yielding Method for Isolation of Genomic DNA from *Ammi majus, Int. Res. J. Biological Sci.,* **2(1,)**, 57-60 **(2013)**