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GENETIC DIVERSITY OF EDIBLE MUSHROOM *PLEUROTUS* SPP. REVEALED BY RANDOMLY AMPLIFIED POLYMORPHIC DNA FINGERPRINTING

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Abstract

The Oyster mushroom (*Pleurotus*) cultivation is a profitable agribusiness and having high significance due its nutritive and therapeutic value. Due to deficient knowledge on *Pleurotus* mushroom genetics seven strains of Oyster mushroom, two local and five exotic were studied for their genetic diversity through RAPD markers. It was clear from similarity matrix that similarity index ranges from 45 to 72%. The cluster analysis of combined data set of all the markers resulted in three major clades, while isolate P-17 remains ungrouped and shown to be the most diverse strain of the seven. During amplification of genomic DNA yielded 70 fragments that could be scored, of which 41 were polymorphic, with an average of 2.73 polymorphic fragments per primer. Number of amplified fragments with random primers ranged from three to six. Polymorphism ranged from 0% to 83.33%, with an overall 58% polymorphism. The allele frequency of RAPD primers ranged from 0.71 to 1.00 while the polymorphic information content highest for the primer GL-C-20 (0.29) followed by the primers GL A-20 and GL C-16 that is zero, indicating medium level of polymorphism among the strains of Oyster mushroom. The objective of the study was to characterize *Pleurotus* strains collected from different origins and to find out the variability at molecular level.

Key words: Diversity, *Pleurotus*, RAPD, Mushroom.

Introduction

Pleurotus ostreatus (Jacq.Fr.) (Oyster mushroom) has high nutritional value and probiotic properties, therefore recommended to be used in diet plan all over the world (Rajewska & Bałasińska, 2004; Florczak *et al*., 2004; Khan *et al*., 2012). Mushrooms contain about 1.5-6.7% carbohydrate, 1.5-3% protein, 0.3-0.4% fats, and vitamins (Bernas *et al.,* 2006; Haq *et al.,* 2010). Rapid increase in the population inadequate provision of nutrients particularly the protein is at alarming situation. Solution of the problems is to explore untraditional sources of protein production. The mushroom cultivation seems to be the most potential alternate source of food to meet this challenge. This fungus also produces important secondary metabolites with pharmaceutical usage and some proteins of potential industrial applications (Wasser, 2010).Mushrooms are useful against diabetes, ulcer, lung diseases, and a excellent antitumor agent (Jose and Janardhanan, 2000). Uses of mushrooms as food supplements, food additives, and in pharmaceutical industry will be increased due to their haematological, antibacterial, antiviral, and antioxidant activity (Yang *et al.,* 2002; Ribeiro *et al*., 2006; Rigula & Siwulski, 2007).Moreover, mushroom cultivation also becomes desirable regarding the problem of waste management, because industrial revolution has resolved the problem by creating a variety of new types of wastes (Anthony, 1977).

DNA finger printing has evolved as a major tool in mushroom characterization (Shinwari *et al.,* 1994; 1994a). The development of RAPD markers has allowed the repaid generation of reliable reproducible DNA fragments in a wide variety of species mushroom. Study of the variation within filamentous fungi has been limited due to lack of useful markers (Crowhurst *et al.,* 1991; Pervaiz *et al.,* 2010; Jan *et al.,* 2011). RAPD technique based on random genomic studies therefore, well-suited to indicate over all genetic variation than sequence analysis of a single region of genome (Achenbach *et al.,* 1996; Akbar *et al.,* 2011). RAPD markers were used for discrimination of various mushroom cultivars and recommended that RAPD markers could assist mushroom strains identification and helpful in protection of elite strains (Moore *et al.,* 2001; Ravash *et al.,* 2009; Agarwal *et al.,* 2013; Rehman *et al.,* 2015). This assay is rapid, independent of gene expression and proves to be beneficial for the grouping of isolates of fungus.

The objective of this study is to characterize local and exotic sp. of oyster mushroom, through RAPD analysis. Little work on *Pleurotus* genetic diversity is available and is a pioneer attempt in Pakistan. Proper characterization of *Pleurotus* sp. is prerequisite for correct identification so that their full potential can be exploited in food sector (Pawlik *et al.,* 2012).Genetic studies are limited due to lack of knowledge about the details of the organization of its genetic material. It is unfortunate that especially in Pakistan mushroom business could not be flourished at large scale, perhaps the reason is lack of mushroom availability at low prices and lack of knowledge.

Material and Methods

Collection of *Pleurotus* sp.: In the present studies seven strains of Oyster mushroom were collected. Two were local i.e., *Pleurotus florida* (P-17) and *Pleurotus ostreatus* (P-19), available in the department of plant pathology. Five exotic strains i.e., *Pleurotus cystidiosus* (wc-609), *Pleurotus (florida) ostreatus* (wc-536), *Pleurotus (flabellatus) djamor* (R-22), *Pleurotus ostreatus* (wc-522) and *Pleurotus (sajor-caju) pulmonarius* (wc-537) were procured from the Culture Bank of the Mushroom Laboratory, Pennsylvania State University, USA (Table 1).

Table 1. *Pleurotus* sp. used in the study.

Sample No.	Strains	Origin
1.	*Pleurotus florida*P-17	Local
2.	*Pleurotus ostreatus* P-19	Local
3.	*Pleurotus (flabellatus) djamor* R-22	Exotic
4.	*Pleurotus cystidiosus*WC-609	Exotic
5.	*Pleurotus (sajor-caju) pulmonarius*WC-537	Exotic
6.	*Pleurotus (florida) ostreatus* WC-536	Exotic
7.	*Pleurotus ostreatus* WC-522	Exotic

Comparative yield of *Pleurotus* sp.: These *Pleurotus* strains were evaluated for their yield on different substrate. Yield data was recorded in different flushes on the basis of total number of fruiting bodies, weight of each fruiting body, and size of fruiting body.

Molecular characterization: *Pleurotus* sp. gave significantly different response in terms of yield, and were further tested for their diversity study on genetic basis by using RAPD markers. For this genomic DNA from young fruiting bodies was extracted by freeze-drying and following protocol was adopted for DNA extraction. A few mycelia threads were multiplied in 1.5 ml eppendorf tubes containing 500µl of liquid potato dextrose agar medium and allowed to grow for 72hrs at 25ºC.The mycelial mat was centrifuged for 5 min at 10,000 rpm, washed with 500µl of TE buffer and pelleted. The buffer was decanted and 300µl of extraction buffer (200 mMtrisHCl, pH 8.5, 250 mM NaCl, 25 mM EDTA 0.5% SDS) was added. The Mycelium of each isolate was crushed with a conical grinder for few min following the addition of 150µl of 3M sodium acetate (pH 5.2). Tubes were placed at -20°C for 10 min and centrifuged. The supernatant was transferred to another tube. An equal volume of isopropanol was added. After 5 min, the precipitated DNA was pelleted by centrifugation. The pellet was washed with 70% ethanol, vacuum dried and dissolved in 20µl TE buffer. The concentration of total genomic DNA was measured by DNA Quant flourometer (Hoefer Dyna Quant TM 200, San Francisco, USA).

Fifteen RAPD primers (Table 2) were used in polymerase chain reaction (PCR). The bands were counted and data were analyzed using Popgen 32 software, version 1.44 (Yeh *et al*., 2000) and the genetic variation and similarity were assessed. The genetic relationship between the *Pleurotus* sp. was determined by clustering the genotypes. All visible and unambiguously scorable fragments amplified by primers were scored under the heading of total scorable fragments.

Results

The variation among different strains of *Pleurotus ostreatus* was first recorded on the basis of yield. Strain WC-536 gave maximum yield on different substrate followed by strain WC-522. Strain R-22 gave the minimum yield among all the tested strains on various substrates. The genomic DNA of seven *Pleurotus ostreatus* strains was analyzed using fifteen 15 RAPD decamers (Tables 1 and 2). The primers were selected for their tendency to yield clear amplification and distinct variation in their banding patterns, indistinct bands were ignored. The variations in the bands showed the profiles based on the primer and the kinds of species tested. Taking into account all the primers and mushroom strains, a total of 70 bands were amplified during PCR. Out of these 41 bands was polymorphic exhibiting overall 58% of polymorphism. The amplification of bands ranged from 250 to 3 kbp in size and 3 to 6 in numbers, with an average of 4.66 bands per primer. Primers GL B-06 and GL C-20 produced maximum polymorphic bands (5 out of 6) with 83% of polymorphism, followed by primers GL A-14 and GL C-18 which produced polymorphic bands (4 out of 6 and 4 out of 5 respectively) with 66% and 80% of polymorphism respectively. Primers GL A-10, GL B-14, GL C-01, GL C-05, GL C-07, and GL C-13 produced three polymorphic bands, with percentage polymorphism of 75%, 75%, 75%, 50%, 60%, and 50% respectively. Primers GL A-02 and GL A-05 produced only two polymorphic bands out of five and four bands, showing 40% and 50% of polymorphism respectively. Primer GL A-11 produced only single polymorphic band out of three bands which showed 33% polymorphism. Primers GL A-20 and GL C-16 failed to produce a polymorphic band (Fig. 1).

The number of amplified bands among different *Pleurotus ostreatus* strains varies from 51 to 60 with an average of 56 bands per *Pleurotus* strains. Strain WC-536 produced the maximum number of amplification (60 bands), followed by WC-522, WC-537, P-17, R-22, and P-19 which produced 59, 58, 57, 55, 53 bands respectively. Strain WC-609 produced the minimum number of amplifications i.e., 51 bands (Fig. 2).

Multivariate analysis was conducted to generate a similarity matrix based on Nei's UPGMA to estimate genetic distance and relatedness of *Pleurotus ostreatus* strains. It was clear form similarity matrix that most closely related strains were P-17 and P-19, and P-17 and WC-609 which showed 72% similarity between them in similarity matrix. Strains R-22 and WC-609 showed 71% similarity between them, followed by strains WC-522 and WC-609 (70% similarity), WC-537 and WC-609 (69% similarity). Strains R-22 and WC-609 showed 68% similarity with strain P-19. Minimum similarity has been observed between the strains WC-522 and R-22 (45%) (Table 3). The Average allele frequency was 0.84 that ranged from minimum 0.71 by marker GL C-20 to maximum 1.00 by marker GL A-20 and GL C-16. The PIC (polymorphic information content) value for 15 RAPD markers ranged from 0 from marker GL A-20 and GL C-16 to 0.29 from marker GL C-20 with an average of 0.18 per marker. The dendrogram produced on the basis of similarity matrix forms different clades. Clade A comprised of two exotic strains WC-609 and WC-522, clade B comprised of R-22 and WC-537. Clade C consists of local strain i.e., P-19 and exotic strain WC-536. Strain P-17 did not form any type of cluster in dendrogram (Fig. 3).

Table 2. Detail of RAPD primers along with their sequences used in the study.

Sr. No.	Primer code	Primer sequence	Allele frequency	PIC value
1.	GL DecamerA-02	TGCCGAGCTG	0.89	0.13
2.	GL DecamerA-05	AGGGGTCTTG	0.89	0.16
3.	GLDecamer A-10	GTGATCGCAG	0.79	0.24
4.	GLDecamer A-11	CAATCGCCGT	0.90	0.11
5.	GL DecamerA-14	TCTGTGCTGG	0.74	0.24
6.	GL DecamerA-20	GTTGCGATCC	1.00	0.00
7.	GL DecamerB-06	TGCTCTGCCC	0.81	0.23
8.	GLDecamerB14	TCCGCTCTGG	0.79	0.23
9.	GL DecamerC-01	TTCGAGCCAG	0.82	0.22
10.	GL Decamer C-05	GATGACCGCC	0.81	0.19
11.	GL DecamerC-07	GTCCCGACGA	0.83	0.19
12.	GL DecamerC-13	AAGCCTCGTC	0.83	0.17
13.	GL DecamerC-16	CACACTCCAG	1.00	0.00
14.	GL DecamerC-18	TGATGGGTG	0.77	0.26
15.	GL DecamerC-20	ACTTCGCCAC	0.71	0.29

Table 3. Similarity matrix for Nei and Li's coefficient of *Pleurotus* sp. obtained through RAPD markers.

Strains	P-19	WC-609	WC-536	P-17	R-22	WC-522	WC-537
P-19	****	0.6812	0.5797	0.7246	0.6812	0.6511	0.6412
WC-609		****	0.5507	0.7246	0.7101	0.7002	0.6910
WC-536			****	0.5942	0.6667	0.5462	0.5341
P-17				****	0.6377	0.6269	0.6172
R-22					****	0.4591	0.4721
WC-522						****	0.4831
WC-537							****

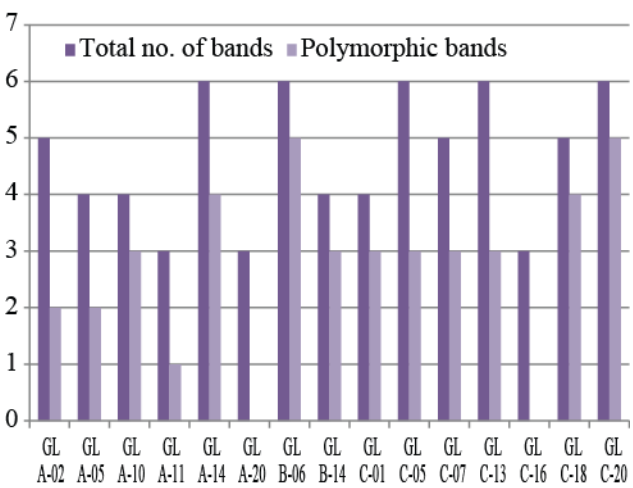

Fig. 1. Number of polymorphic bands per primer.

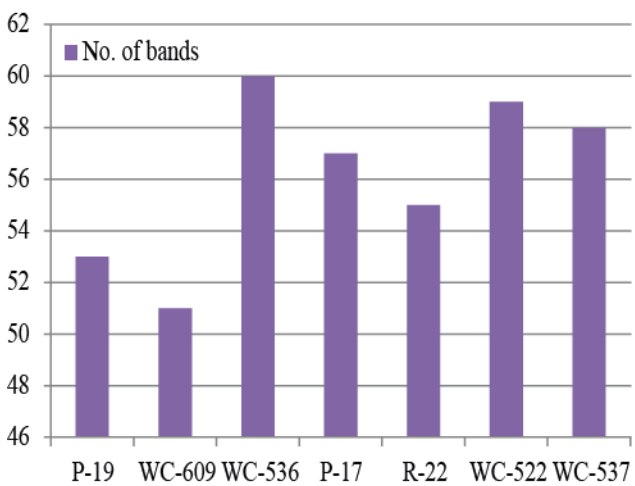

Fig. 2. Number of bands per genotype.

Discussion

Variation among *Pleurotus* strains was studied on the basis of yield potential, size of fruiting body, etc. Morphological traits do not provide a meaningful framework of evolutionary classification (Rabbani *et al.*, 2010). Within the slightly simple fruiting structures and considerable developmental plasticity of fungi, it is accepted that variations in developing sub-populations are not always expressed in terms of morphological divergence. Closely related species therefore, lack taxonomically useful morphological differences. Significant problems in classifying *Pleurotus* on the basis of morphological traits were already observed (Fonseca *et al.*, 2008). The basic DNA sequence presumed to be insensitive to short term environmental change and thus should provide a more stable alternative for strain discrimination.

As genetic diversity studies on mushroom have been reported to a limited extend, therefore *Pleurotus* strains were examined for their diversity by using RAPD primers. Genetic diversity was observed within the

Pleurotus strains and results were in confirmation with the findings of Shukla and Jaitly, 2011. High polymorphism 94%-99% was observed in genetic diversity of *Pleurotus* strains (Yin *et al.,* 2013; Lewinsohn *et al*., 2001) as compared to present study. Whereas polymorphism observed in current study (58%) was similar as observed by Fu *et al.,* 2010 during the genetic diversity studies of *Lentinula edodes*. Average number of bands amplification per *Pleurotus* strain was 56 which were greater as compared to bands observed by Stajic *et al.* (2005).The bands produced in current study were similar in size range as given by Alam *et al.* (2009).The average number of bands per primer produced in current study were higher as compared to Chandra *et al*. (2010). However *Pleurotus* strains diversity by using RAPD markers was found to be 36-46%, which showed more diversity than present study results. Results of the similarity matrix (72%) obtained in current study matched with the findings of Chandra *et al*. (2010). Yadav *et al*. (2007) observed polymorphic information content ranged 0.00 to 2.12 from RAPD markers. The PIC value obtained in this study ranged from 0.00 to 0.29 showed medium level of polymorphism as described Qiu *et al.,* 2013. More number of clades of *Pleurotus* strains belonging to different geographical region was found (Zervakis *et al.,* 2001; Stajic *et al.,* 2005; Wang *et al.,* 2012; Yin *et al.,* 2013) as compared to present study. In current study three clades were produced showing more diversity between the exotic and local strains of *Pleurotus*. So, a strong correlation was observed between the strains genetic makeup and their origin. Current DNA finger printing results will be used in a database for inter laboratory use and future reference to be used in breeding commercial strains. In future microsatellite data can be used to further clarify the questions raised (Turi *et al.,* 2012).

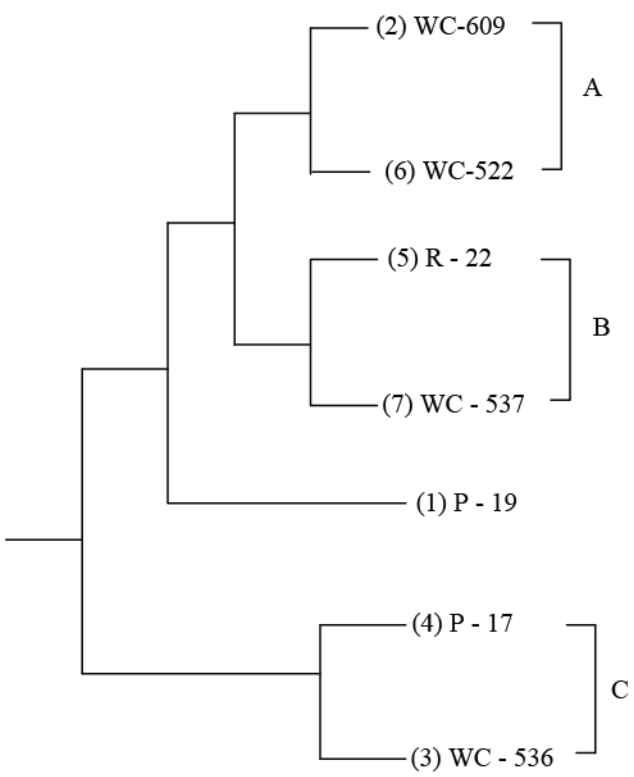

Fig. 3. Dendrogram of *Pleurotus* sp. developed from similarity matrix.

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