RESEARCH ARTICLE

Pleurotus species as a source of nutraceuticals including vitamin B_{12} and lignocellulosic degradative enzyme

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ABSTRACT

In the present study, we collected *Pleurotus* isolates from diverse edaphic zones from the parts of Uttar Pradesh. Neutraceuticals (proteins, carbohydrates, phenolic, vitamin B_{12}) and xenobiotics degradation capacity of textile dyes along with the production of laccase enzyme evaluated. Isolate no. 06 appeared most distant in the dendrogram showing the highest MG degradation capacity, however, others showed excellent degradation of BPB. The laccase enzyme activity was found in the range of 4.03 to 19.13 IU/ml from mycelia extract. Gene frequency within isolates from 0.012 to 0.987 was analyzed through RAPD and the average gene diversity for all loci was 0.244 and the Shannon Information Index was found 0.397. The unbiased genetic similarity among isolates was 0.36 to 0.93 with a mean of 0.64. Significant genetic diversity, nutraceuticals and laccase enzyme availability and dye degradation capacity of the studied genus *Pleurotus*, was found, which makes it necessary to carry out a selection process in each one for superior selection not only for human being but also many aquatic as well as other terrestrial flora and fauna. The present investigation first time reported that *Pleurotus* species as a source of vitamin B_{12} in range of 0.05 to 0.32 mg/kg (of dried mushroom).

Key words: bromophenol blue, genetic diversity, malachite green, *Pleurotus* species, vitamin B_{12}

Introduction

The genus *Pleurotus* belongs to class Basidiomycetes (Castellano, 1989), is one of the most important edible mushrooms and has many bio-potentialities right from nutritive mushroom production to bio-degradative non-specific extracellular enzyme production. This genus has a pool of non-specific enzymes (e.g. laccases, polyphenol oxidase, xylanase) that help in the efficient colonization and decomposition of a variety of lignocellulosic materials. These mushrooms play a role in the degradation of xenobiotic compounds due to many non-specific extracellular enzymes (Hofrichter, 2002; Royse, 2002; Rajak et al., 2011; Young et al., 2015; Loi et al., 2016). Along with these lignocellulosic degradative properties, it also has many bio-potentialities applicable in environmental and biotechnological applications (Rajarathnam et al., 1992, 1998; Rajarathnam and Bano, 1989; Vishwakarma et al., 2012). It also plays a critical role in human health, agriculture and food industry and as model organisms for basic scientific studies (Patel et al., 2012, 2017; Valverde et al., 2015).

Traditionally, edible species of the genus *Pleurotus* considered as medicinally important mushrooms (Bano and Rajarathnam, 1988; Khan and Tania, 2012) due to their properties like antibacterial, antiviral, immunomodulatory, hypocholesterolemic, anticholesterolic, antimutagenic, hyperglycemic, etc. as reviewed by many researchers (King, 1993; Gregori et al., 2007; Patel et al., 2012; Valverde et al., 2015). Along with medicinal properties, it also has many important nutritional components like vitamins, fibres, proteins and essential amino acids and low cholesterols (Mattila et al., 2001). With medicinal and nutritional values, it is considered as “nutraceuticals” (Chang and Buswell, 1996).

Currently, commercial mushroom production is based on the limited number of strains available, which are at high risk of environmental changes, hence, genetic diversity is critical for adaptation to environmental changes and for the long-term survival of the species. Knowledge of genetic diversity within and among populations has practical importance for conservation and management policies (Hamrick and Godt, 1989; Fritsch and Rieseberg, 1995). The preservation of genetic diversity within the species is a major target of conservation because the loss of genetic variation is thought to reduce the ability of populations to adapt to environmental change for survival (Hogbin and Peakall, 1999; Parmesan, 2006; Fisher et al., 2012). Therefore, population genetic studies of *Pleurotus* spp are essential for providing necessary information for the conservation of this very important genus.

PCR-based DNA fingerprinting techniques such as Random Amplified Polymorphic DNA analysis (RAPD), Inter Simple Sequence Repeat (ISSR) and Amplified Fragment Length Polymorphism (AFLP) represent a very informative and cost-effective approach for assessing genetic diversity for a wide range of organisms. All these markers do not require any prior knowledge of the genome of the species (Williams et al., 1990; Zietkiewicz et al., 1994; Bornet and Branchard, 2001). RAPD has been the most employed technique due to its simplicity and fast (Howell et al., 1994). Despite questions about its reproducibility, its utility in diversity analysis, mapping and genotype identification has been exploited in plant and fungi (Jones et al., 1997; Chandra et al., 2010).

The species diversity of genus *Pleurotus* at the DNA level is completely lacking in the studied area. This study by the strain identification and genetic analysis of the *Pleurotus* spp could help to uncover novel, economically important genetic variations for breeding purposes and removal of pollutants from water bodies and agro-industrial waste materials. Studying the genetic diversity using RAPD markers provide an opportunity to scan the entire genome for direct comparison of genetic materials that is almost independent of environmental influences (Harvey and Botha, 1996; Bautista et al., 2003; Zhao and Pan, 2004). This is the first study on the inter-population genetic diversity of *Pleurotus* in this diverse geographical region. The objectives of the present work were: (i) collection, purification and evaluation of bio-potentialities of isolates of *Pleurotus* from different edaphic zones, (ii) textile dye degradation potential of the collected isolates and (iii) genetic variability study via RAPD of the collected isolates.

Materials and Methods

Sample collection

Sixty isolates of genus *Pleurotus* were collected as per the standard protocol for macrofungi outlined by Mueller et al. (2004) from the six identified geo-ecological zones of eastern Uttar Pradesh (India) namely: *usar*, wastelands, forest area, wetlands, flood area and fertile lands (Table 1); out of which, thirty-nine isolates could be preserved for further studies. The collection of isolates was accompanied by recording of many morphological features as given in Table 2. Remaining characteristics such as size and color of basidiospores were recorded in the laboratory after the cultivation of purified isolates. Pure culture of the isolates was finally preserved on potato dextrose agar (PDA) slants and stored at 4°C for further availability.

Characterization of isolates through PCR-fingerprinting

Genomic DNA was isolated from mycelia of pure cultures by the CTAB method (Sadowsky et al., 1987; Kuramae, 1997). Only high-quality DNA (260/280 = 1.7-1.9) were used in this study. Genetic diversity within collected isolates was characterized by PCR (Williams et al., 1990) using arbitrary 10-mer primers as given in the Table 3.

Table 1. *Details of geographic coordinates of studied districts with their geographical characters, in which one district could be comprises of more than one eco-edaphic zones.*

Geo-ecological zones	Districts	Altitude (m)	Latitude	Longitude
Usar	Jaunpur	79.5 – 88.3	24°24′N-26°12′N	82°70′E-83°50′E
	Allahabad	72.0 – 98.0	24°87′N-25°27′N	81°51′E-82°51′E
	Bhadohi	85.0 – 88.5	25°24'N-25°42'N	82°38'E-82°57'E
Foresting area	Mirzapur	80.0 – 128.9	23°32′N-25°52′N	82°7′E-83°53′E
Wetlands	Allahabad	72.0 – 98.0	24°87′N-25°27′N	81°51′E-82°51′E
	Varanasi	75.7 – 80.7	25°14′N-25°23.5′N	82°56′E-83°8′E
	Jaunpur	79.5 – 88.3	24°24′N-26°12′N	82°70′E-83°50′E
	Mirzapur	80.0 – 128.9	23°32′N-25°52′N	82°7′E-83°53′E
Flood area	Jaunpur	79.5 – 88.3	24°24′N-26°12′N	82°70′E-83°50′E
	Allahabad	72.0 – 98.0	24°87′N-25°27′N	81°51′E-82°51′E
	Varanasi	75.7 – 80.7	25°14′N-25°23.5′N	82°56′E-83°8′E
	Azamgarh	64.0 – 71.5	25°90′N-26°38′N	82°40′E-83°53′E
Fertile lands	Allahabad	72.0 – 98.0	24°87′N-25°27′N	81°51′E-82°51′E
	Azamgarh	64.0 – 71.5	25°90′N-26°38′N	82°40′E-83°53′E
	Varanasi	75.7 – 80.7	25°14′N-25°23.5′N	82°56′E-83°8′E
	Jaunpur	79.5 – 88.3	24°24′N-26°12′N	82°70′E-83°50′E
	Mirzapur	80.0 – 128.9	23°32′N-25°52′N	82°7′E-83°53′E
Wastelands	Mirzapur	80.0 – 128.9	23°32′N-25°52′N	82°7′E-83°53′E
	Allahabad	72.0 – 98.0	24°87′N-25°27′N	81°51′E-82°51′E
	Varanasi	75.7 – 80.7	25°14′N-25°23.5′N	82°56′E-83°8′E

Table 2. *Morphological features of basidiocarps (fruiting bodies), their pileus, stipe, gills and spores of collected isolates of Pleurotus species.*

	Basidiocarp		Pileus (cap)				Stipe				Gills				Spore	
S.N.	Habit	Shape	Size cm (h×d)	Surface	Margin	Colour	Size (h×d)	Shape	Localization	Texture	Attachment	Spacing	Width (mm)	Edge	Size (µ)	Colour
1	Solitary	Concave	20.0×12.0	Scaly	Incurred	Brown	1.3× 3.2	Cyl*	Lateral	Fleshy	Decurrent	Distant	5	Entire	11.7×4.3	Cream-white
2	Solitary	Concave	5.0× 3.5	Waxy	Incurred	Dull-white	1.3× 3.2	Cyl	Lateral	Fleshy	Decurrent	Crowded	3	Entire	35.0×5.0	White
3	Clustered	Concave	7.3×6.2	Scaly	Straight	White	0.8×0.5	Cyl	Lateral	Rigid	Adnexed	Distant	2	Entire	7.0× 10.0	White
4	Solitary	Concave	10.0×7.0	Scaly	Incurred	Dull-white	3.5×1.5	Cyl	Lateral	Fleshy	Decurrent	Crowded	4	Entire	7.5×3.0.75	White
5	Clustered	Concave	9.5×6.5	Smooth	Incurved	Pale-white	1.2×1.4	Bulb	Lateral	Rigid	Free	Crowded	3	Entire	15.0×4.0	White
6	Clustered	Bell-shaped	3.5×4.0	Scaly	Incurved	White	9.5×1.0	Cyl	Central	Brittle	Adnexed	Crowded	1	Entire	22.5×5.0	White
7	Solitary	Knobbed	4.0×6.0	Smooth	Incurved	Dull-white	1.0×1.0	Tap**	Lateral	Fleshy	Adnate	Crowded	2	Entire	25.2×7.0	White
8	Clustered	Flat	5.1×5.6	Velvety	Straight	Pale-white	3.0×1.7	Tap	Lateral	Fleshy	Free	Distant	9	Entire	11.0×3.5	White
9	Clustered	Bell-shaped	4.1×5.8	Rough	Incurved	Pale-white	2.0×0.5	Cyl	Central	Fleshy	Decurrent	Crowded	1	Entire	15.0×4.5	White
10	Clustered	Bell-shaped	4.1×5.8	Rough	Enrolled	Dull-white	3.0×0.5	Cyl	Lateral	Fleshy	Decurrent	Crowded	1	Entire	22.0×4.5	White
11	Clustered	Funnel	5.5×6.5	Scaly	Incurred	Dull-white	1.5×3.7	Cyl	Central	Rigid	Decurrent	Crowded	2	Entire	17.0×4.8	White
12	Solitary	Knobbed	3.2×3.5	Shiny	Straight	White	1.0×0.5	Tap	Lateral	Rigid	Adnexed	Crowded	2	Entire	21.0×5.0	White
13	Solitary	Flat	7.5×4.5	Smooth	Straight	Pale-white	1.7×1.5	Tap	Lateral	Rigid	Adnate	Crowded	3	Entire	30.5×12	White
14	Solitary	Concave	5.1×5.0	Smooth	Straight	White	1.5×0.9	Cyl	Lateral	Rigid	Adnexed	Distant	3	Entire	26.8×7.8	White
15	Clustered	Convex	10.0×9.0	Smooth	Straight	White	8.0 ×1.0	Cyl	Lateral	Fleshy	Adnate	Crowded	3	Entire	14.5×9.5	White
16	Clustered	Flat	5.4×4.1	Smooth	Straight	White	1.5×0.8	Cyl	Lateral	Fleshy	Adnate	Crowded	3	Entire	8.8×3.75	White
17	Solitary	Concave	2.9×4.1	Smooth	Incurved	Milky-white	0.9×1.1	Bulb	Lateral	Fleshy	Free	Crowded	3	Entire	41.2×9.8	White
18	Solitary	Convex	6.6×4.4	Scaly	Straight	Dull-white	1.0×7.0	Tap	Lateral	Rigid	Adnate	Crowded	1.8	Entire	51.0×5.0	White
19	Solitary	Convex	4.2×3.5	Velvety	Incurved	White	2.0×1.0	Tap	Lateral	Rigid	Free	Distant	5	Entire	18.5×5.6	White
20	Clustered	Concave	7.5×8.0	Smooth	Incurved	Brown	0.9×1.0	Cyl	Lateral	Fleshy	Free	Crowded	3	Entire	9.8×4.75	White
21	Solitary	Flat	5.0×3.5	Smooth	Incurved	White	0.8×0.8	Bulb	Lateral	Rigid	Adnexed	Distant	3	Entire	15.8×6.8	White
22	Solitary	Concave	13.5×9.0	Smooth	Incurved	Pale-white	5.0×1.5	Cyl	Lateral	Fleshy	Adnexed	Distant	1.4	Entire	41.0× 6.5	White
23	Solitary	Concave	5.5×8.3	Smooth	Enrolled	Creamy	0.8×1.1	Tap	Lateral	Rigid	Adnexed	Distant	5	Entire	21.0×6.5	White
24	Solitary	Convex	6.1×7.5	Smooth	Straight	Dull-white	3.0×1.1	Tap	Lateral	Rigid	Free	Distant	7	Entire	34.0×4.0	White
25	Clustered	Bell-shaped	7.1×6.5	Scaly	Incurved	White	3.5×0.7	Tap	Lateral	Rigid	Adnate	Crowded	3	Entire	46.0× 14.0	White
26	Solitary	Knobbed	4.1×3.9	Velvety	Incurved	White	2.9×1.1	Cyl	Lateral	Fleshy	Adnexed	Distant	7	Entire	31.2×12.8	White
27	Clustered	Knobbed	6.1×4.8	Smooth	Incurved	Dull-white	4.0×1.0	Cyl	Lateral	Fleshy	Decurrent	Distant	3	Entire	7.5×10.2	White
28	Clustered	Vas-shaped	8.0×7.0	Smooth	Enrolled	Brown	2.5×1.8	Cyl	Lateral	Fleshy	Adnexed	Crowded	2	Entire	9.8×7.75	White
29	Clustered	Vas-shaped	8.0×7.0	Smooth	Enrolled	Brown	2.5×1.8	Cyl	Lateral	Fleshy	Adnexed	Crowded	2	Entire	45.7×10.8	White
30	Clustered	Convex	5.5×5.0	Smooth	Incurved	White	2.0×1.0	Cyl	Lateral	Fleshy	Decurrent	Crowded	3	Entire	17.0×3.8	White
31	Clustered	Convex	5.5×5.0	Smooth	Incurved	Pink	0.6×0.8	Bulb	Lateral	Fleshy	Decurrent	Crowded	3	Entire	25.4×6.8	White
32	Solitary	Vas-shaped	6.5×6.5	Smooth	Enrolled	Brown	1.8×1.5	Cyl	Lateral	Fleshy	Adnexed	Crowded	4	Entire	23.5×3.5	White
33	Clustered	Convex	5.1×5.6	Smooth	Incurved	Pale-white	1.0×0.3	Cyl	Lateral	Fleshy	Decurrent	Crowded	1	Entire	7.9×10.2	White
34	Clustered	Convex	9.5×6.0	Smooth	Incurved	White	0.5×1.0	Bulb	Lateral	Fleshy	Decurrent	Crowded	4	Entire	5.5×3.5	White
35	Solitary	Convex	4.1×3.6	Velvety	Straight	Dull-white	2.0×1.7	Tap	Lateral	Fleshy	Free	Crowded	3	Entire	10.8×3.75	White
36	Solitary	Flat	6.0×7.0	Smooth	Incurved	Dull-white	1.5×4.0	Cyl	Lateral	Fleshy	Decurrent	Crowded	4	Entire	19×7.4	White
37	Scattered	Concave	5.0×7.0	Velvety	Incurved	White	1.8×1.4	Tap	Lateral	Fleshy	Adnexed	Crowded	3	Entire	6.3×10.4	White
38	Clustered	Concave	9.5×6.0	Smooth	Incurved	White	0.5×1.0	Bulb	Lateral	Fleshy	Decurrent	Crowded	4	Entire	26×14.2	White
39	Solitary	Concave	5.6×4.3	Waxy	Straight	Dull-white	4.5×1.2	Cyl	Lateral	Fleshy	Decurrent	Crowded	3	Entire	38.2×9.8	White

Legend: ** Cyl – cylindrical, ** Tap – tapering*

Amplification was carried out in biological triplicate in a thermal cycler (Bio-Rad) in 25 µl reaction volume containing DNA (40-100 ng), 1X *Taq* buffer, 10mM dNTPs, primer (10 pM) and 1-2 units of *Taq* DNA polymerase with the negative control. Amplification reaction included i) initial denaturation at 95°C for 5 min, ii) 35 cycles of denaturation at 94°C for 60 sec, primer annealing at ~32 °C (varied with primers) for 30 sec and DNA synthesis at 72°C for 150 sec; and iii) final amplification at 72°C for 10 min. Amplified products were resolved on 1.2% agarose gel along with a 100 bp DNA ladder. The gels were observed under the Gel-Doc system (Alfa Imager) and pictured images used for further scoring of the banding patterns in different isolates.

Table 3. *Primers with their sequence, GC% and annealing temperature.*

Primers	Sequence (5`→3`)	GC%	Annealing temperature (°C)
B-78	CTGCTGGGAC	70%	33.6
B-76	GTTTCGCTCC	60%	33.7
B-75	AGCTGACCGT	60%	32.6
B-73	TCCGCTCTGG	70%	40.3
B-77	TGATCCCTGG	60%	33.9
B-74	CCACAGCAGT	60%	28.6
B-71	GAAACGGGTG	60%	33.6
B-72	GTGACGTAGG	60%	33.7
B-79	GGGTAACGCC	70%	41.9
B-80	GTGATCGCAG	60%	33.9

Quantification of nutraceuticals

The total protein content in dried fruiting bodies was analyzed by the standard method of Bradford (Bradford, 1976) and total carbohydrate content available in mycelium was determined using the phenol-sulfuric acid method of Dubois et al. (1956), while, the total amount of phenolics content of methanolic extract of dried mycelia was measured as per standard method developed by Singleton et al. (1965).

Bioassay of Vitamin B_{12}

Bioassay of vitamin B_{12} was conducted based upon the methods of AOAC (USP, 28, NF, 23, 2005). In which, Vitamin B_{12} assay medium (minimal medium) for the growth of test organism (*Lactobacillus delbrueckii* subsp. *lactis*) except vitamin B_{12}. The assay medium was prepared in double strength and 5 ml medium was taken in each test tube (Made: Borosil) to which increasing amounts of a standard solution of or the unknown & sufficient water was added to give a total volume of 10 ml per tube. All the tubes were sterilized for 5 min at 10 psi and immediately cooled at room temperature. 100 µl of the inoculums (fresh culture of *Lactobacillus delbrueckii* subsp. *lactis*) were inoculated into each of the assay tubes. The growth of bacteria in the assay medium was measured at 530 nm by spectrophotometer (1 cm path length) after incubation for 36 h at 37 ±1 °C in a shaker.

Laccase enzyme activity

Laccase activity was determined via the oxidation of Guaiacol (o-methoxy phenol catechol monomethyl ether) as a substrate as per the method used by Arora and Sandhu (1985) and details given by Patel et al. (2015). The enzyme extracts were prepared by homogenization of mycelial mat in a buffer in cold condition and then the activity was calculated as per the formula: IU/ml= ΔA@470nm/0.001

Dye decolorization

To investigate the decolorization potential of the isolates, the two most used dyes- Bromophenol Blue (BPB) and Malachite greenG (MG) were procured from Himedia Biosciences (India) and were evaluated on solid medium. It was based on the measurement of the bleached area by mycelia growth on solid medium either supplemented with test dyes or without dye as control (Machado et al., 2006). For decolorization assay, PDA plates were prepared with 0.01% and 0.05% (w/v) of MG and BPB supplementation, respectively. Point inoculation was performed on both types of the plate as mycelial plugs prepared from pure mycelial mother plate with the help of cork-borer and kept at the center of the PDA plates which were incubated at 28°C±1 in BOD incubator. The clear zone was measured under and around the developing mycelia from the center of the plates (plate diameter: 90.0 mm). Results were observed after 5 and 10 days, the clear zone appeared against the blue background. The experiments were performed in biological triplicates.

Statistical analysis

Analysis of mean SD and SE were calculated by Microsoft excel. However, analysis of variability and allele frequency, allele number, effective allele number, polymorphic loci, observed homozygosity, expected homozygosity, Shannon Index (Gillies, 1997), Gene diversity, neutrality test (Manly, 1985) and unbiased genetic distance were calculated by POPGENE 32 software. Pair-wise genetic dissimilarities among the isolates were calculated from the binary data using well known Jaccard's Coefficient to form the matrix of genetic dissimilarities. The dendrogram was obtained by using NTSYS-PC software version 2.02j through the RAPD binary matrix from which cluster analysis was performed by means of unweighted pair group method using arithmetic average (UPGMA). The variability among the isolates was assessed by comparing RAPD fragments according to their sizes and the presence/absence of shared fragments. All the statistical analyses related to nutritional elements were conducted using the SPAR v. 2.0.

Results

Collection and purification of isolates

Six eco-edaphic zones identified in the present study as given in the Table 1 and depicted in Figure 1 were thoroughly observed and identified in the rainy season for the hotspot of *Pleurotus* mushroom. Isolates were collected from the habituated dead and decayed mango (*Mangifera indica* Linn.) trees. A total of 39 isolates were purified out of 60 collected isolates from different zones and their morphological features (Table 2) were observed during and after collection.

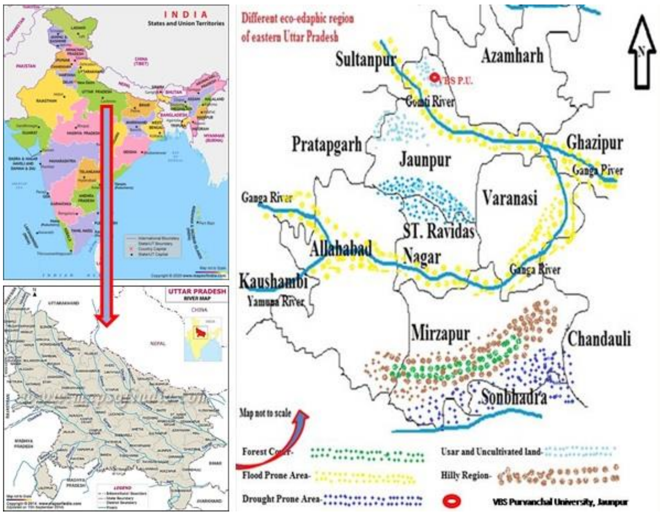

Figure 1. *Eco-edaphic zones of selected area under investigation for the study of genetic diversity of Pleurotus species. Enlarge map of selected area from the State depicted right side of the figure. Dots in different color represent five different zones and rest white background represents fertile land. Arrow in the upper right most corner indicates direction north. There is two major rivers (Ganga and Yamuna) and many small rivers (Varuna, Gomti, Sayi, etc.)*

Interestingly, it was observed from Table 2 that mixed types of *Pleurotus* were collected from different zones; which indicates that studied areas are diverse rich. Fruiting bodies were found both clustered as well as solitary types, however, the shape of basidiocarps were diverse ranging from concave, bell-shaped, funnel-shaped, convex, knobbed, Vas-shaped to flat having scaly, waxy, velvety, rough or even smooth surface with varying in the area ranging from 8.12 cm^2 to 240 cm^2. Fruiting bodies of collected isolates were of various colors such as brown, white, milky white, pink; which demonstrated the importance of the study of diversity through the collection of natural isolates for further strain improvements and betterment of various potentialities. Stipes of isolates were found very interesting having majority in fleshy nature, however, few of them were with rigid stipe. The majority of stipes were cylindrical in shape, some of them were in tapering and few were in bulb shape. There was also diversity in the attachment of stipes from its pileus, the majority of them were in lateral, which is a signature characteristic of Oyster mushrooms, however, few of them were centrally attached with its pileus. Gills found under the pileus were majorly in crowded with few distantly located having an entire completed edge, some of them were serrated. Spores collected from fruiting bodies cultivated in the lab were white in color and varying in size.

DNA polymorphism through RAPD profiling and their allelic frequency

For the study of DNA polymorphism, genomic DNA from all the isolates was subjected to RAPD based DNA profiling to detect variability in the genome. Ten single strand primers (arbitrary primers) were chosen for amplification. The sizes of amplification products varied from 100-2500 bp and the degree of polymorphism depended upon the isolate and the primer employed. For convenience, the results of RAPD profiling were broadly categorized into three groups as given in Table 4 depending upon the degree of polymorphism as evident from the banding pattern of amplified products on agarose gels. Out of the 10 selected primers used in this study, 6 of them amplified the DNA and generated 51 polymorphic bands given in Table 4. The results clearly indicate that primers- B-73, B-74, B-75, B-76, B-77 and B-78 produced 8, 6, 9, 10, 6, and 12, respectively discrete and scorable bands in all isolates. Primer B-78 amplified the highest number of scorable bands (i.e. 12), whereas, primer B-74 and B-77 amplified the lowermost number of bands. Each sample was characterized by a different RAPD genotype. For the assessment of genetic diversity of isolates, the frequencies of all resolved RAPD alleles (e.g. from B-73, B-74, B-75, B-76, B-77 and B-78) were calculated as given in Table 5. The allele 1 having the highest frequency has great importance in diversity study.

Table 4. *Different primers and their polymorphism status.*

Polymorphism	Primer	Sequence (5`→3`)	Bands
High	B-78	CTGCTGGGAC	12
	B-76	GTTTCGCTCC	10
	B-75	AGCTGACCGT	9
	B-73	TCCGCTCTGG	8
Moderate	B-77	TGATCCCTGG	6
	B-74	CCACAGCAGT	6
Zero	B-71	GAAACGGGTG	1
	B-72	GTGACGTAGG	1
	B-79	GGGTAACGCC	1
	B-80	GTGATCGCAG	1

Genetic diversity among isolates

Many descriptive measures of diversity are calculated for the estimation of diversity among collected isolates from different ecological zones as described by Nei (1973).

Table 5. *Frequency of genes/alleles in isolates.*

Locus	Alleles		Locus	Alleles	
	Allele 1	Allele 0		Allele 1	Allele 0
B73-1	0.152	0.847	B76-4	0.302	0.698
B73-2	0.122	0.877	B76-5	0.215	0.784
B73-3	0.152	0.847	B76-6	0.152	0.847
B73-4	0.094	0.905	B76-7	0.232	0.767
B73-5	0.167	0.832	B76-8	0.232	0.767
B73-6	0.152	0.847	B76-9	0.094	0.905
B73-7	0.026	0.974	B76-10	0.012	0.987
B73-8	0.137	0.862	B77-1	0.232	0.767
B74-1	0.066	0.933	B77-2	0.108	0.891
B74-2	0.215	0.784	B77-3	0.039	0.960
B74-3	0.122	0.877	B77-4	0.199	0.800
B74-4	0.167	0.832	B77-5	0.052	0.947
B74-5	0.094	0.905	B77-6	0.012	0.987
B74-6	0.094	0.905	B78-1	0.026	0.974
B75-1	0.094	0.905	B78-2	0.137	0.862
B75-2	0.232	0.767	B78-3	0.152	0.847
B75-3	0.248	0.751	B78-4	0.199	0.800
B75-4	0.167	0.832	B78-5	0.266	0.733
B75-5	0.137	0.862	B78-6	0.183	0.816
B75-6	0.283	0.716	B78-7	0.152	0.847
B75-7	0.400	0.599	B78-8	0.183	0.816
B75-8	0.167	0.832	B78-9	0.183	0.816
B75-9	0.066	0.933	B78-10	0.039	0.960
B76-1	0.012	0.987	B78-11	0.122	0.877
B76-2	0.422	0.577	B78-12	0.108	B78-12
B76-3	0.199	0.800			

These descriptive measures are: number of loci analyzed, percent polymorphic loci, mean number of alleles per locus, gene diversity, the effective number of alleles (Kimura and Crow, 1964), observed heterozygosity and Shannon`s Information Index (Lewontin, 1972) as given in Table 6. The isolates were assigned to 39 different subtypes by RAPD analysis. The average gene diversity (as per Nei`s) for all RAPD loci was 0.24±0.12. The minimum gene diversity was shown by *B76*-1 loci, i.e. 0.02 while maximum diversity was shown by *B76*-2 loci, i.e. 0.488. The observed number of alleles was 2.00±0.00 while the effective number of alleles was 1.35±0.21 (Table 6). Shannon`s Information Index was found to 0.40±0.15. Nei's (1978) unbiased genetic similarity among all pairs of samples is given in the Table 7. This similarity in matrix clearly showed 11% (min) and 84% (max) similarity between isolates #7 and #8. The Ewens-Watterson test for neutrality for each locus was calculated by POPGENE as given in the Table 8 showed that the allele frequencies at all loci were selectively neutral in the studied isolates. Where for each observed allele frequency, upper (U95) and lower (L95) were at 95% confidence limits of expected F values.

Clustering of isolates based on DNA profiles

Dendrogram, as illustrated in the Figure 2, was generated by Nei's genetic distance given in the Table 7. Each leaf represents an individual observation. The leaves are spaced

Table 6. *Summary of gene diversity for all RAPD's loci as per Nei (1973).*

Locus	Sample	Number of alleles		Gene diversity (h)	Shannon's index (I)
		Observed (na)	Effective (ne)		
B73-1	39	2.0000	1.3491	0.2587	0.4273
B73-2	39	2.0000	1.2749	0.2157	0.3727
B73-3	39	2.0000	1.3491	0.2587	0.4273
B73-4	39	2.0000	1.2057	0.1706	0.3121
B73-5	39	2.0000	1.3879	0.2795	0.4526
B73-6	39	2.0000	1.3491	0.2587	0.4273
B73-7	39	2.0000	1.0533	0.0506	0.1205
B73-8	39	2.0000	1.3114	0.2375	0.4007
B74-1	39	2.0000	1.1413	0.1238	0.2440
B74-2	39	2.0000	1.5109	0.3382	0.5212
B74-3	39	2.0000	1.2749	0.2157	0.3727
B74-4	39	2.0000	1.3879	0.2795	0.4526
B74-5	39	2.0000	1.2057	0.1706	0.3121
B74-6	39	2.0000	1.2057	0.1706	0.3121
B75-1	39	2.0000	1.2057	0.1706	0.3121
B75-2	39	2.0000	1.5538	0.3564	0.5417
B75-3	39	2.0000	1.5973	0.3739	0.5612
B75-4	39	2.0000	1.3879	0.2795	0.4526
B75-5	39	2.0000	1.3114	0.2375	0.4007
B75-6	39	2.0000	1.6852	0.4066	0.5966
B75-7	39	2.0000	1.9243	0.4803	0.6734
B75-8	39	2.0000	1.3879	0.2795	0.4526
B75-9	39	2.0000	1.1413	0.1238	0.2440
B76-1	39	2.0000	1.0261	0.0255	0.0690
B76-2	39	2.0000	1.9533	0.4880	0.6811
B76-3	39	2.0000	1.4689	0.3192	0.4995
B76-4	39	2.0000	1.7289	0.4216	0.6126
B76-5	39	2.0000	1.5109	0.3382	0.5212
B76-6	39	2.0000	1.3491	0.2587	0.4273
B76-7	39	2.0000	1.5538	0.3564	0.5417
B76-8	39	2.0000	1.5538	0.3564	0.5417
B76-9	39	2.0000	1.2057	0.1706	0.3121
B76-10	39	2.0000	1.0261	0.0255	0.0690
B77-1	39	2.0000	1.5538	0.3564	0.5417
B77-2	39	2.0000	1.2397	0.1934	0.3433
B77-3	39	2.0000	1.0815	0.0754	0.1655
B77-4	39	2.0000	1.4689	0.3192	0.4995
B77-5	39	2.0000	1.1109	0.0998	0.2063
B77-6	39	2.0000	1.0261	0.0255	0.0690
B78-1	39	2.0000	1.0533	0.0506	0.1205
B78-2	39	2.0000	1.3114	0.2375	0.4007
B78-3	39	2.0000	1.3491	0.2587	0.4273
B78-4	39	2.0000	1.4689	0.3192	0.4995
B78-5	39	2.0000	1.6412	0.3907	0.5794
B78-6	39	2.0000	1.4279	0.2997	0.4767
B78-7	39	2.0000	1.3491	0.2587	0.4273
B78-8	39	2.0000	1.4279	0.2997	0.4767
B78-9	39	2.0000	1.4279	0.2997	0.4767
B78-10	39	2.0000	1.0815	0.0754	0.1655
B78-11	39	2.0000	1.2749	0.2157	0.3727
B78-12	39	2.0000	1.2397	0.1934	0.3433
Mean	**39**	**2.0000**	**1.3551**	**0.2444**	**0.3972**
SD Dev		**0.0000**	**0.2148**	**0.1159**	**0.1547**

evenly along the horizontal axis. The vertical axis indicates a distance or dissimilarity measure. The height of a node represents the distance of the two clusters that the node joins. The obtained dendrogram depicts that all isolates fall into two distinct groups (similarity >12%). Similarity indices were developed on the basis of amplified fragments of the 39 different genotypes using 6 RAPD primers (Table 4). The genetic similarity values ranged from 0.36 to 0.93 with a mean of 0.64.

Table 7. *Nei's unbiased measures of genetic similarity (Nei, 1978).*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.45	1.00																		
3	0.20	0.25	1.00																	
4	0.45	0.63	0.30	1.00																
5	0.20	0.30	0.45	0.30	1.00															
6	0.14	0.27	0.18	0.21	0.18	1.00														
7	0.52	0.63	0.25	0.63	0.17	0.16	1.00													
8	0.55	0.59	0.22	0.66	0.22	0.12	0.84	1.00												
9	0.16	0.14	0.36	0.14	0.15	0.04	0.18	0.14	1.00											
10	0.23	0.38	0.54	0.44	0.41	0.33	0.33	0.29	0.28	1.00										
11	0.27	0.33	0.25	0.33	0.30	0.20	0.28	0.29	0.07	0.33	1.00									
12	0.22	0.27	0.52	0.42	0.34	0.11	0.32	0.28	0.43	0.48	0.26	1.00								
13	0.20	0.21	0.39	0.30	0.45	0.13	0.25	0.17	0.25	0.30	0.30	0.45	1.00							
14	0.23	0.24	0.38	0.19	0.38	0.21	0.14	0.15	0.35	0.34	0.23	0.28	0.26	1.00						
15	0.27	0.23	0.30	0.18	0.30	0.04	0.23	0.19	0.27	0.14	0.33	0.26	0.42	0.28	1.00					
16	0.17	0.10	0.31	0.14	0.26	0.00	0.19	0.15	0.42	0.29	0.23	0.33	0.38	0.44	0.35	1.00				
17	0.23	0.20	0.30	0.28	0.25	0.07	0.28	0.25	0.39	0.24	0.18	0.37	0.30	0.24	0.18	0.29	1.00			
18	0.17	0.24	0.20	0.19	0.31	0.04	0.19	0.20	0.23	0.19	0.35	0.33	0.38	0.36	0.28	0.36	0.29	1.00		
19	0.27	0.18	0.25	0.23	0.15	0.09	0.28	0.24	0.27	0.23	0.16	0.22	0.11	0.23	0.07	0.17	0.60	0.12	1.00	
20	0.16	0.26	0.19	0.22	0.10	0.08	0.32	0.28	0.16	0.17	0.11	0.17	0.03	0.12	0.11	0.07	0.32	0.07	0.52	1.00
21	0.21	0.18	0.25	0.18	0.15	0.04	0.33	0.24	0.21	0.18	0.12	0.17	0.15	0.12	0.21	0.17	0.39	0.12	0.55	0.61
22	0.10	0.18	0.14	0.08	0.20	0.20	0.04	0.04	0.10	0.18	0.22	0.12	0.09	0.40	0.15	0.10	0.13	0.23	0.15	0.21
23	0.08	0.20	0.21	0.20	0.21	0.15	0.11	0.11	0.18	0.20	0.18	0.24	0.16	0.38	0.18	0.13	0.20	0.25	0.18	0.28
24	0.13	0.30	0.16	0.20	0.16	0.17	0.15	0.16	0.18	0.11	0.18	0.19	0.07	0.19	0.23	0.04	0.11	0.16	0.18	0.35
25	0.17	0.24	0.16	0.19	0.16	0.09	0.19	0.20	0.12	0.19	0.23	0.14	0.11	0.23	0.28	0.18	0.10	0.18	0.08	0.16
26	0.09	0.12	0.18	0.03	0.18	0.06	0.07	0.08	0.14	0.03	0.14	0.16	0.13	0.15	0.33	0.21	0.23	0.23	0.14	0.16
27	0.14	0.23	0.28	0.15	0.28	0.11	0.18	0.12	0.36	0.25	0.17	0.25	0.28	0.26	0.33	0.37	0.32	0.32	0.22	0.20
28	0.12	0.19	0.12	0.19	0.26	0.09	0.19	0.20	0.12	0.14	0.23	0.14	0.26	0.23	0.28	0.23	0.24	0.36	0.08	0.07
29	0.23	0.24	0.25	0.16	0.17	0.03	0.24	0.20	0.33	0.24	0.18	0.27	0.17	0.24	0.23	0.34	0.28	0.29	0.23	0.32
30	0.12	0.24	0.21	0.10	0.45	0.09	0.14	0.15	0.12	0.19	0.28	0.23	0.31	0.36	0.28	0.30	0.24	0.52	0.12	0.12
31	0.23	0.14	0.15	0.18	0.25	0.15	0.17	0.11	0.12	0.10	0.28	0.10	0.16	0.18	0.28	0.18	0.24	0.23	0.28	0.12
32	0.12	0.14	0.15	0.10	0.15	0.09	0.14	0.14	0.21	0.06	0.21	0.13	0.20	0.17	0.21	0.23	0.18	0.23	0.12	0.07
33	0.13	0.07	0.08	0.07	0.17	0.10	0.07	0.07	0.08	0.07	0.25	0.07	0.17	0.26	0.25	0.20	0.16	0.33	0.13	0.04
34	0.04	0.03	0.23	0.00	0.18	0.11	0.00	0.00	0.14	0.12	0.14	0.16	0.08	0.15	0.09	0.09	0.07	0.09	0.09	0.13
35	0.21	0.12	0.13	0.08	0.08	0.00	0.17	0.13	0.15	0.08	0.15	0.07	0.08	0.04	0.21	0.15	0.12	0.04	0.21	0.14
36	0.15	0.17	0.13	0.17	0.19	0.00	0.23	0.18	0.04	0.12	0.27	0.21	0.13	0.04	0.21	0.10	0.12	0.10	0.15	0.14
37	0.08	0.15	0.16	0.11	0.12	0.04	0.20	0.1	0.08	0.11	0.18	0.19	0.16	0.04	0.23	0.08	0.07	0.04	0.13	0.28
38	0.20	0.17	0.29	0.13	0.14	0.25	0.17	0.10	0.20	0.32	0.26	0.13	0.19	0.27	0.26	0.21	0.13	0.07	0.16	0.15
39	0.16	0.13	0.24	0.10	0.14	0.19	0.13	0.10	0.11	0.26	0.11	0.09	0.06	0.21	0.07	0.16	0.22	0.12	0.26	0.20

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
21	1.00																		
22	0.10	1.00																	
23	0.18	0.53	1.00																
24	0.13	0.33	0.33	1.00															
25	0.08	0.17	0.25	0.39	1.00														
26	0.14	0.05	0.04	0.22	0.35	1.00													
27	0.30	0.12	0.18	0.18	0.22	0.25	1.00												
28	0.12	0.10	0.25	0.08	0.23	0.15	0.37	1.00											
29	0.28	0.23	0.36	0.25	0.29	0.21	0.35	0.14	1.00										
30	0.12	0.23	0.19	0.13	0.30	0.27	0.32	0.36	0.19	1.00									
31	0.17	0.10	0.19	0.25	0.23	0.35	0.22	0.30	0.19	0.18	1.00								
32	0.12	0.10	0.13	0.13	0.28	0.20	0.36	0.42	0.14	0.28	0.28	1.00							
33	0.08	0.26	0.15	0.21	0.33	0.16	0.24	0.26	0.07	0.33	0.33	0.31	1.00						
34	0.09	0.20	0.22	0.22	0.27	0.11	0.15	0.09	0.21	0.21	0.09	0.26	0.23	1.00					
35	0.21	0.00	0.00	0.16	0.22	0.26	0.31	0.10	0.28	0.04	0.22	0.21	0.11	0.11	1.00				
36	0.15	0.06	0.10	0.16	0.22	0.26	0.11	0.15	0.23	0.15	0.22	0.15	0.05	0.11	0.38	1.00			
37	0.23	0.11	0.09	0.26	0.31	0.29	0.23	0.08	0.20	0.13	0.08	0.23	0.15	0.29	0.31	0.40	1.00		
38	0.16	0.21	0.23	0.28	0.27	0.19	0.20	0.12	0.32	0.12	0.16	0.11	0.18	0.31	0.20	0.14	0.28	1.00	
39	0.16	0.21	0.17	0.17	0.21	0.19	0.25	0.16	0.37	0.12	0.21	0.11	0.13	0.19	0.33	0.20	0.23	0.30	1.00

All isolates diverged into two major clusters, represented as I and II (Figure 2) except isolate #06 that clustered separately from all the isolates. The first (cluster-I) major cluster was divisible into two sub-clusters (A & B) at 18% similarity level, in which one sub-cluster (i.e. sub-cluster-A) comprised of three isolates (#35, #36, #37) while the other sub-cluster (sub-cluster-B) again divisible into two sub-sub-cluster (a & b), each comprised four isolates (sub-sub-cluster a- #29, #39, #34, #38 and sub-sub-cluster b- #22, #23, #24, #25).

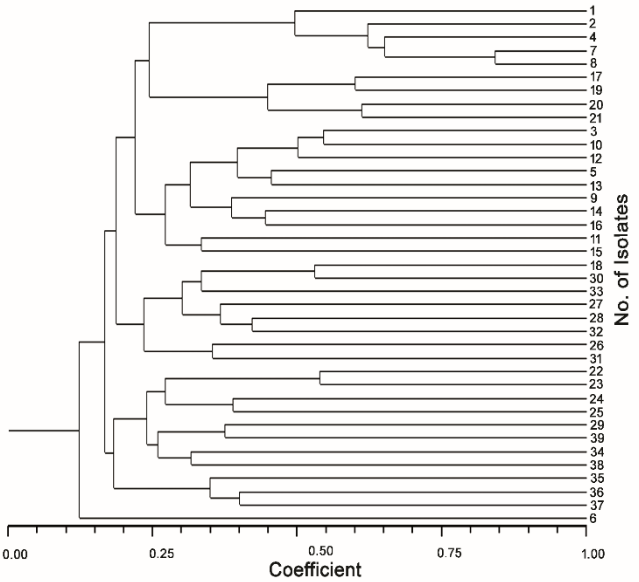

Figure 2. *Dendrogram obtained from the matrix of RAPD through different primers used in the study and prepared by NTSYS software. There is Nei`s coefficient on x-axis, representing the similarity amongst the isolates, however, on the Y-axis, no of isolates given.*

Second major cluster (cluster-II) was divisible into three sub-clusters (A, B & C). Sub-cluster-A comprised eight isolates (#31, #26, #32, #28, #27, #33, #30 & #18), sub-cluster-B comprised ten isolates (#15, #11, #16, #14, #9, #13, #5, #12, #10 & #3), while sub-cluster-C further divisible into two sub-sub-clusters-a & b. Sub-sub-clusters-a comprised four isolates (#21, #20, #19 & #17), while sub-sub-clusters-b comprised five isolates (#8, #7, #4, #2 and #1).

Availability of nutraceuticals

Total crude protein quantified in dried fruiting bodies of a different isolate of *Pleurotus* species is given in Figure 3. The highest amount of protein was observed in #29 i.e., 28.48 mg/100mg when compared with 39 isolates; and the lowest content is in #31 with a value of 17.16 mg/100mg. The amount of protein present in any strain is dependent on many factors basically types of substrate and other additional ingredients of substrate including weather conditions such as temperature. The total carbohydrate value was calculated and found in the range from 3.55 to 5.43 g/100g of fresh oyster mushroom as given in Figure 4. It was observed that the highest carbohydrate content (5.43g/100g) was found in #10, however, lowest in #18. The total phenolic content was determined in the fresh fruiting body of isolates of *Pleurotus* collected in the present study and draw a bar diagram as depicted in Figure 5. The content of phenolics was different in different isolates and it was in the range of 21.19 to 36.32 mg/g, in which #21 showed maximum and #01 showed minimum phenolic content of in their fruiting body.

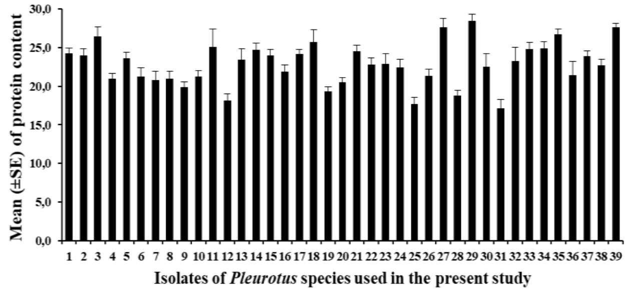

Figure 3. *Content of total proteins measured by Lowry et al. (1951) method.*

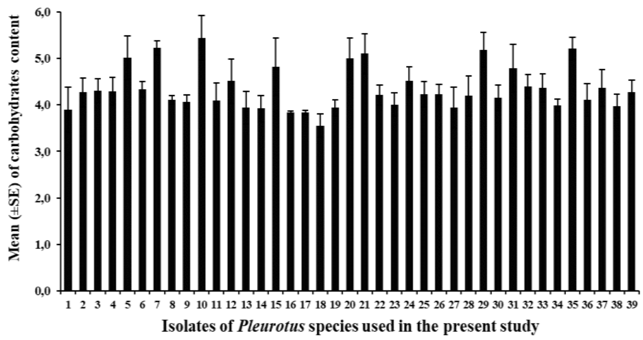

Figure 4. *Content of total carbohydrates measured by phenol sulphuric acid method.*

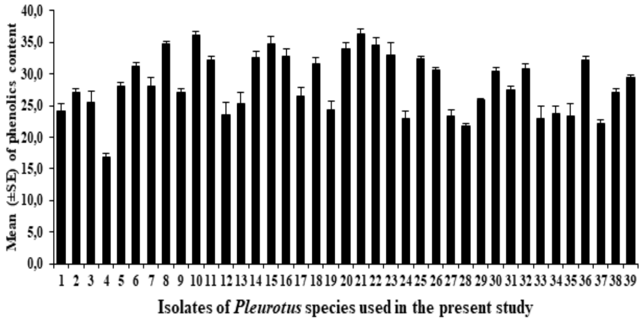

Figure 5. *Content of total phenolic measured by Singleton et al. (1965) method.*

Vitamin B_{12} content of different isolates was determined by bioassay with vitamin B_{12} requiring microorganisms, such as *Lactobacillus delbrueckii* subsp. *lactis* as described by Schneider (1987). A good amount of vitamin B_{12} was observed, which was in the range of 0.05 to 0.32 mg/kg (of dried mushroom) of vitamin B_{12} in different isolates of

Pleurotus spp as given in Figure 6. The highest amount of vitamin B_{12} is observed in isolate #32 i.e., 0.32 mg/kg.

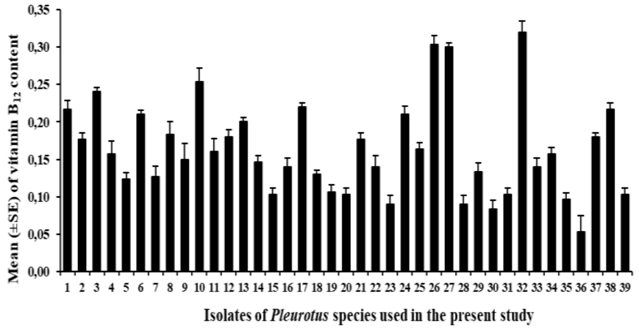

Figure 6. *Vitamin B12 contents in fruiting bodies of different isolates of Pleurotus species.*

Laccase enzyme

Production of fungal laccase was assayed via the oxidation of Guaiacol (o-methoxy phenol catechol monomethyl ether) as a substrate as per the method used by Arora and Sandhu (1985) by the extracellular enzyme obtained through liquid state fermentation from growing mycelia of different isolates of *Pleurotus* spp. Figure 7 showed that #2 produced maximum and #27 minimum laccase enzyme i.e., 4.03 and 19.13 IU/ml, respectively.

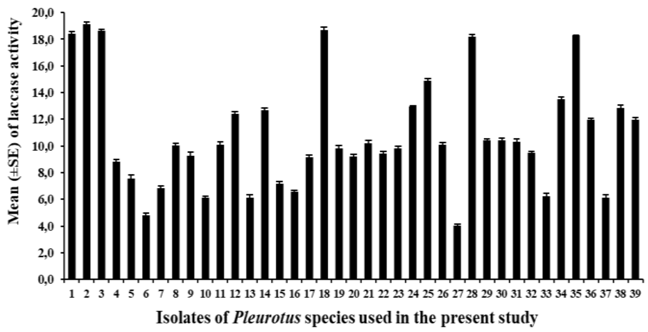

Figure 7. *Laccase enzyme activity assayed by oxidation of Guaiacol (o-methoxyphenol catechol monomethylether) as substrate as per the method used by Arora and Sandhu (1985).*

Decolorization of malachite greenG (0.01%) and bromophenol blue (0.05%)

The *Pleurotus* isolates were analyzed for their decolorizing ability of two textile dyes i.e., malachite greenG (MG) and bromophenol blue (BPB) on solid medium. The isolates were able to decolorize MG (in terms of decolorized area) within 5-10 days of incubation at 28±1°C when PDA was supplemented with 0.01% (w/v) of MG. From the observation of Figure 8, most of the isolates were not efficient to complete the decolorization of MG. Only isolate #06 showed the highest ability of degradation of MG dye (>75%), however, rest isolates showed lower capacity in decolorizing of MG. Similarly, decolorization potential for BPB was observed as given in Figure 9. The dye degradation capacity of isolates was higher in bromophenol blue while moderate and lower was observed in case of malachite greenG (Table 9).

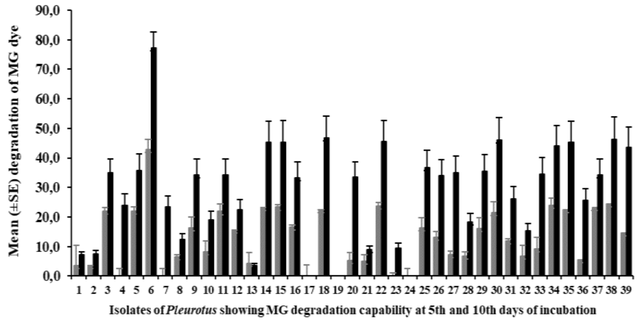

Figure 8. *Isolates of Pleurotus species showing decolorization of malachite greenG (0.01% (w/v) concentration at 5th day and 10th day of incubation at 28±°C.*

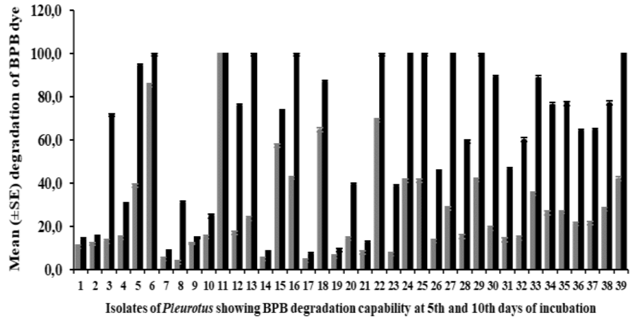

Figure 9. *Isolates of Pleurotus species showing decolorization of Bromophenol blue (0.05% (w/v) concentration at 5th day and 10th day of incubation at 28±°C.*

Discussion

In the present study, RAPD markers showed effective discrimination of closely related species of *Pleurotus* (Figure 2), out of 10 arbitrary primers, 6 primers exhibited a total of 51 polymorphic bands as given in Table 4, and all were 100% polymorphic. The number of amplified products varied depending upon the primers used; primers B-73, B-74, B-75, B-76, B-77 and B-78 generated 8, 6, 9, 10, 6, and 12 bands, respectively. These variations in the number of bands may be due to the sequence of primer, availability of annealing sites in the genome and template quality (Kernodle et al., 1993). Afzal et al. (2004) reported only 75% polymorphism while studying 21 cultivars of mungbean employing 34 RAPD primers (8.5 bands per primer), while, 73.2% polymorphism was reported in *Gymnema sylvestre* with 15 RAPD primers (Nair and Keshavachandran, 2006), with 6.0 bands per primer, however, Yin et al. (2012) reported a still higher average value (about 13.7). Unlike MLEE, RAPD analyses generally detect the occurrence of a single allele that assesses polymorphisms at a wide range of loci (Williams et al., 1990). Later, many researchers (Dowdy and McGaughey, 1996; Pornkulwat et al., 1998) differentiated very closely related species or even geographical populations using RAPDs. The use of RAPDs provided a much clearer picture of the relationships between Pleurotus species and studied eco-geographic zones. Gene frequency was found in the range of 0.012 to 0.987, in which the highest gene frequency was 0.422 of allele-1 for B76-2 loci while the lowest gene frequency was 0.012 of allele-1 for B77-6 loci. Alleles having a frequency of less than 0.01 were not detected. In certain pairs of alleles (e.g. B73-2, B74-3 & B78-2; B76-3 & B77-4) frequencies of alleles are uniform that may indicate the active participation of natural selection in maintaining genetic polymorphisms as discussed.

Table 9. *The three classes of degradation capacity of dyes: highly efficient (>50%), medium (50-30%) and lower in efficiency (<30%) degradation of MG and BPB of isolates of Pleurotus species.*

Classes	Bromophenol Blue (BPB)		Malachite greenG (MG)	
	5th day	10th day	5th day	10th day
High	#6, #11, #15, #18, #22	#3, #5, #6, #11, #12, #13, #15, #16, #18, #22, #24, #25, #27, #28, #29, #30, #32, #33, #34, #35, #36, #37, #38, #39	–	#6
Moderate	#5, #16, #24, #25, #29, #33, #39	#4, #8, #20, #23, #26, #31	#6	#3, #5, #9, #11, #14, #15, #16, #18, #20, #22, #25, #26, #27, #29, #30, #33, #34, #35, #37, #38, #39
Low	#1, #2, #3, #4, #7, #8, #9, #10, #12, #13, #14, #17, #19, #20, #21, #23, #26, #27, #28, #30, #31, #32, #34, #35, #36, #37, #38	#1, #2, #7, #9, #10, #10, #14, #17, #19, #21	#1, #2, #3, #4, #5, #7, #8, #9, #10, #11, #12, #13, #14, #15, #16, #17, #18, #19, #20, #21, #22, #23, #24, #25, #26, #27, #28, #29, #30, #31, #32, #33, #34, #35, #36, #37, #38, #39	#1, #2, #4, #7, #8, #10, #12, #13, #17, #19, #21, #23, #24, #28, #31, #32, #36

In order to understand the genetic diversity more critically, more biometric parameters like the effective number of alleles, Nei`s (1973) genetic distance and Shannon`s information index were also calculated and given in Table 6. Genetic diversity was found from 0.025 to 0.488 whereas, the mean 0.244±0.116; which indicates that many of the loci differ between all pairs of RAPD genotypes. Lowest gene diversity was shown by *B76-1* loci with a value of 0.025 while the highest was by *B76-2* loci showing a value of 0.488. These results suggested that the RAPD approach showed considerable potential for *Pleurotus* species discrimination. These results are similar to Yin et al. (2012) who reported substantial genetic diversity (0.22 to 0.97) amongst 15 different cultivars of *P. pulmonarious* using RAPD. Similar results (genetic diversity: 0.178-0.262) were obtained by Zervakis et al. (2001) while studying genetic polymorphism in *P. eryngii* species complex growing in the greater Mediterranean area. In the present study, the observed number of alleles was 2.00±0.00 while the effective number of alleles was 1.35±0.21 as given in Table 6; the Shannon`s Information Index varied from 0.12 to 0.68 with an average 0.40±0.15. These results are in agreement with Boldo et al. (2003) who made epidemiological studies with 47 clinical and reference strains of *Candida glabrata* from several geographical origins. The value of this index is related to the diversity of isolates, value > 0.5 is considered to be higher diversity. Coefficients of genetic similarity were calculated from a paired comparison of the all 39 isolates, based on the normalized identity of each locus in each of species (Nei, 1978), was in the range from 0.11 to 0.84 similar to the results of Chandra et al. (2010).

So far the statistical analyses of data obtained from RAPD profiling are in the form of loci and alleles. Therefore, any polymorphism between two samples based upon the RAPD marker is the manifestation of polymorphism of its loci and alleles. It might be expected that RAPD markers would relate with eco-edaphic zones of the studied area, from where isolates collected. However, it is to be noted that RAPDs produce dominant markers, and in addition, it scans different parts of the DNA generating a large number of markers, and hence, different results may be obtained, such as a RAPD marker reveals the nucleotide differences in a random sequence of DNA of 10 bases long (if a decamer is used). The estimation of genetic variation by this method, as performed in this study with RAPD, permits better estimations of genetic diversity from any species as compared to morphological and physiological parameters.

In order to see the relationship amongst the isolates used in this study, a dendrogram was generated from the pairwise distance matrices. The clustering pattern in Jaccard’s similarity, dendrogram generated by RAPD as given in Figure 2 demonstrated the discriminating power of RAPDs with reference to their eco-geographic zones. Similar results were obtained by Liu and Furnier (1993) in a study of the genetic variation in aspen (*Populus* spp.), and by Lanner-Herrera et al. (1996) who studied the diversity in natural populations of wild kale, *Brassica oleracea* L. Dendrogram (Figure 2) generated through RAPD data from UPGMA cluster analysis demonstrated that all isolates clustered into two distinct groups in the distance 0.12. All isolates fell into two major clusters except isolate #06 that come into viewed separately from all the clustered isolates; all these were grouped into seven clusters.

On the basis of allelic frequencies of RAPDs loci, isolates were separated into seven clusters. At the distance of 0.12, most isolates formed clusters and they are further grouped as discussed earlier. The first cluster mainly comprised five isolates (#1, #2, #4, #7, #8,), second cluster comprised four isolates (#17, #19, #20, #21), third cluster comprised ten isolates (#3, #10, #12, #5, #13, #9, #14, #16, #12, #15), fourth cluster comprised eight isolates (#18, #30, #33, #27, #28, #32, #26, #31), fifth cluster comprised eight isolates (#22, #23, #24, #25, #29, #39, #34, #38), sixth cluster comprised only three isolates (#35, #36, #37) while single isolate (#6) represented seventh cluster. However, it is worth noting that all isolates used in this study almost grouped with reference to their respective eco-geographic zones. Discrimination of isolates by RAPD markers with reference to their respective eco-geographic zones suggested that geographic isolation strongly influenced the evolution of the populations as similarly explained by Sun et al. (1999).

These results indicated some correlation amongst the isolates with respect to their collection site/native place, similar results were observed by Sonnante et al. (1997) who studied the genetic diversity within and between *Vigna luteola* and *V. Marina* (fodder crop). They observed that RAPD markers were able to disclose a much higher level of polymorphisms based upon isozymes profile essentially at the intraspecific level. A possible explanation for the differences found among these dendrograms might be based on the kind of information provided by each type of marker. These RAPDs detect variation in both coding and non-coding regions. Small, repeated, and random sequence mutations would be accumulated in non-coding sequences, and the diversity can be revealed by RAPD. Another factor that needs to be considered for RAPD analysis is that bands of identical mobility may occasionally correspond to non-homologous fragments (Chalmers et al., 1992; Tinker et al., 1993). Although, in an epidemiological study involving pathogenic isolates of *Aspergillus fumigatus*, a dendrogram was prepared using isozyme, and RAPDs were very coherent on the basis of cophenetic analysis (Rinyu et al., 1995). This could be due to the fact that high value of cophenetic correlation coefficient was due to the high number of negative matches, since on use of the Jaccard`s coefficient, which does not take into account the negative matches, however, in another study involving the population of *Elymus caninus* (a species of flowering plant in the Poaceae family) dendrograms derived from isozyme and RAPD data showed no correlation between clusters and geographic origins (Sun et al., 1999).

It is well accepted that the level of genetic variation is generally considered adaptive and related to the breadth of geographical ranges and/or to the ecological heterogeneity within the ranges (Lewinsohn *et al.*, 2000; Nevo, 1988). Speciation and the development of species richness appear to be facilitated by restricted gene flow and isolation of small populations (Lande, 1984). Hence, the high diversity in many intraspecific taxa that are tropically highly specialized suggests that ecologically specialized populations are particularly prone to speciation (Futuyma, 1986a). However, if those populations are brought into contact, much of the divergence they have accomplished will be lost by interbreeding. On the other hand, if they have evolved into a new species they can retain their diverse adaptations, and refine them even while sympatric (Futuyma, 1986b). In many cases, sympatric populations are in an intermediate stage of speciation (i.e. partially reproductively isolated), and they usually interbreed along a hybrid zone that can persist for long periods (Futuyma, 1986a).

The present study was completed in the eastern part of Uttar Pradesh; commonly called Purvanchal comprises of more than fifteen districts including Allahabad, Azamgarh, Jaunpur, Mirzapur, S.R.N. Bhadohi and Varanasi (plus half a dozen more carved out from above districts). The topology of this region is considerably heterogeneous, with a gradient of temperature, precipitation, waterlogging (Puri, 1992) which is more suitable for generating diversity. Long- and short-term environmental factors (e.g. flood, drought and soil erosion) and likely are crucial to the creation and maintenance of high biodiversity (Taylor and Skinner, 1998). The diversity of this region is considered endangered due to the fragmentation of critical habitat (DellaSala et al., 1999). In this study some sort of association was observed with their geographic origin of isolates with RAPD profiling, however less or no association was observed when MLEE was employed as reported by Patel et al. (2017). Environmental and edaphic factors are known to influence the diversity of terrestrial forms in general (Boddy et al., 2013) that also influence the genotype of oyster mushroom fungus in the long run.

The degrees of decolorization of different dyes such as malachite green, indigo carmine, xylidine ponceau, Bismarck brown and methyl orange using the white-rot fungus *P. ostreatus* were previously evaluated by various researchers (Hofrichter, 2002; Rabinovich et al., 2004; Cerniglia and Sutherland, 2010). It was similar as we observed and measured in the present study with MG and BPB decolorization. In this decolorization capacity, laccase (Revankar and Lele, 2007) and MnP (Tsukihara et al., 2008) play a major role in the complete oxidation of dyes (Vishwakarma et al., 2012). These enzymes oxidized in a nonspecific way to both phenolic and nonphenolic lignin derivatives and thus are promising candidates for the degradation of environmental pollutants (Fahr et al., 1999; Ferreira-Leitao et al., 2007; Vishwakarma et al., 2012) and highly recalcitrant compounds such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) and lignin derivatives have been attributed to the oxidative enzymes, especially laccase (Riccardi et al., 2005). Surprisingly, we also assayed laccase concentration in liquid mycelia growth medium and it was in accordance with many previous studies (Inácio et al., 2015; Xie et al., 2016).

Dried fruiting bodies of different isolates contain a sufficient amount of crude protein (Figure 3) and reported by many researchers also (Tolera and Abera, 2017). Similarly, total carbohydrates and phenolic content measured, and found very lower carbohydrates content which makes it a very suitable food for the diabetic patient especially (Parul and Asha, 2014; Widyastuti et al., 2015; Tolera and Abera, 2017). Total phenolics in this study were in range 21.19 to 36.32 mg/g and it was found in agreement with a previous report (Abugri and McElhenney, 2013; Tan et al., 2015). From the Figure 4 it is clear that *Pleurotus* has produced a sufficient quantity of vitamin B_{12} to fulfill the daily requirement of our population. Though adults need only 2.3 to 5.0µg of B_{12} per day for optimum health, dietary intake of B_{12} should exceed that amount due to the complex process required to assimilate and metabolize this essential nutrient. Uptake of this vitamin in the gastrointestinal tract depends on intrinsic factor, which is synthesized by the gastric parietal cells, and on the cubam receptor in the distal ileum (Nielsen et al., 2012). As per guidelines of the Institute of Medicine (USA), consumption of approximately 100 g of dried oyster mushrooms could provide the recommended daily dietary allowance (2.4 µg/day) for adults (Sullivan and Herbert, 1965).

Conclusion

The present study demonstrates that the molecular markers generated through RAPD are more useful as compared to morphological markers for evaluating genetic diversity through characterization and identification of relationships among *Pleurotus* species of mushrooms vis-a-vis geographical zones. It indicated a high level of genetic polymorphism amongst the isolates of *Pleurotus* species despite the availability of a relatively lower number of isolates of *Pleurotus*. The dendrogram based upon RAPDs reflected better geographic affinities that took into account all DNA fragments. Although no evidence of selective effects of any polymorphic loci was recorded in this study because the correlation with climatic and physical variables was non-significant. Hence, the isolates of different zones are meaningfully addressed by the dendrograms obtained from RAPD data, which correlated and discriminated against the eco-geographic group by RAPD markers suggests that geographic isolation may influence the evolution of the populations. The Oyster mushroom studied in terms of diversity was also evaluated for their many potentials including protein, carbohydrates, vitamin B_{12}, laccase enzyme, degradation of textile dyes and phenolic; which showed variability amongst isolates. The results of this study indicate the potential of diversity in terms of natural products.

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