academicJournals

Vol. 7(43), pp. 5057-5065, 25 October, 2013
DOI: 10.5897/AJMR2013.5472
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Morphological, ultrastructural and molecular variability studies of wild and mutant strains of edible *Pleurotus* species using growth yield, scanning electron microscopy and random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)

Ola, I. O.[1,2]*, Unni, B. G.[1], Oloke, J. K.[2] and Bordoloi, A. K.[3]

[1]Biotechnology Division, CSIR-North-East Institute of Science and Technology, Jorhat, Assam, India.
[2]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[3]Medicinal, Aromatic and Economic Plant Division, CSIR-North-East Institute of Science and Technology, Jorhat, Assam, India.

Accepted 22 September, 2013

There is a growing industry of edible mushroom production due to their nutritive value and the recognized fact that mushrooms are natural and healthy foods originating from an environmentally friendly organic farming system. The production of edible mushrooms is threatened by both abiotic and biotic factors, hence the need to improve breeding through genetic tools. In an attempt to determine the morphological and genetic diversity among *Pleurotus* species, fourteen different strains of *Pleurotus ostreatus* and *Pleurotus florida* wild type and their mutants were subjected to different morphological traits, ultrastuctural hyphae network studies and random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) marker. The mycelia growth yield on Petri-plates and in submerged fermentation indicated that the strains PO90 and PF30 were significantly different from the other *Pleurotus* strains used in this study at $P < 0.05$. Also, the microscopy result showed marked differences among the *Pleurotus* strains. The dendrogram based on RAPD analysis generated two different clusters. Out of 4 random primers, distinct polymorphism was observed by primers BG17, BG18, BG 23 and BG25. The percentage similarity among the *Pleurotus* strains varies between 40-100%.

Key words: *Pleurotus* species, submerged fermentation, scanning electron microscopy, biomass yield, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR).

INTRODUCTION

Mushrooms have been consumed by humans since ancient times, not only as a part of the normal diet but also as a delicacy due to their desirable taste and aroma. Mushrooms have been employed for several useful purposes. They have been employed in pharmaceutical, food and agro allied industries (Alofe et al., 1998;

*Corresponding author. E-mail: olaiyabo@yahoo.com. Tel: +2348036843319 or +918011343267.

Adejoye and Fasidi, 2009; Akinfemi, 2009).

Mushrooms are well known for their medicinal properties and have been widely used in traditional medicine. The medicinal effects of mushrooms include antioxidant, antibacterial, antifungal, antiviral, anti-parasitic, antitumor, immunomodulatory, anti-inflammatory, radical scavenging, anti-diabetic and hepato-protective (Wasser, 2010).

The use of mushrooms with therapeutic properties is growing day by day due to the range of side effects caused by conventional medicines. Many, if not all, higher mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelia and cultured broth (Wasser 2010a; Smith et al., 2003).

Mushrooms are currently evaluated for their nutritional value and acceptability as well as for their pharmacological properties. In the developing countries of the world such as Nigeria, uncontrolled population growth has created problem of limited food supply, which has led to search for new methods to provide adequate food for humans. The majority of mushroom lovers in developing countries depend solely on mushrooms that grow in the wild (Jonathan and Adeoyo, 2011).

Edible mushrooms are widely eaten by many Nigerian ethnic groups such as the Hausas and Fulanis in the north, and the Yorubas, Ibos, Urhobos, Ijaws and Itsekiris in the south. In Nigeria, mushroom eating is more popular in the villages than in urban areas because, rural people have access to natural vegetation where mushrooms grow.

The genus *Pleurotus* is a heterogeneous group of economic importance. Several species are of nutritional and/or medicinal importance (Cohen et al., 2002; Guzman, 2000). *Pleurotus* species have the ability to absorb microelements from different cultivation media and thus they may present an excellent dietary source (Stajic et al., 2002).

Fungi of the *Pleurotus* genus have an important place among the commercially employed basidiomycetes because they have gastronomic, nutritional and medicinal properties and can be easily cultivated on a large range of substrates (Chang and Hayes, 1978; Kumari and Achal, 2008).

Oyster mushrooms are a more valuable source of protein than either cattle or fish on dry weight basis, and are a good source of almost all the essential amino acids when compared with most vegetables and fruits (Matila et al., 2002).

However, problems in evaluating data published by different investigators working with even the same species of mushroom exist because identification of species is often not accurate. The correct identification of the taxonomic position of mushroom cultures is a task of vital importance.

Before the introduction of molecular tools of identification, there have been a lot of taxonomic chaos; this was because the traditional morphological studies for identification were not well standardized.

An important aspect of the search for natural products with biotechnological potential is that the correct identification of the source species is determined.

The aim of this research was to carry out a detailed study of morphological growth characteristics, vegetative mycelia structures and genomic DNA phylogeny of different strains of *Pleurotus* isolates showing potential biotechnological application, by assessing the growth yield, phase contrast microscopy, scanning electron microscopy and random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)as an identification tool.

MATERIALS AND METHODS

Microorganisms

The *Pleurotus* species used in this study were obtained from the Mushroom Research Germplasm, CSIR-NEIST, Jorhat, Assam, India. *Pleurotus ostreatus* and *Pleurotus florida* wild-type and their generated mutants were used. The strains were maintained on potato dextrose agar (PDA) slants at 4°C for subsequent uses and sub-cultured bi-monthly.

Mutant strains induction

P. ostreatus and P. florida mutant strains (PO15, PO30, PO45, PO60, PO75, PO90 and PF15, PF30, PF45, PF60, PF75 and PF90) were produced by randomly exposing an actively growing culture (5 days old) of the fungus on PDA plate to an ultraviolet-irradiation (λ = 254 nm) at different time durations of 15 min interval for 90 min (Decla*n* and Alan, 2001). Mycelia plugs obtained from the culture were transferred onto the centre of a fresh PDA plates and incubated at 25°C. After several exposure to UV mutation and sub-culturing of the mutants, mycelia plugs from the survival mutants were selected for further study.

Media

Potato dextrose agar (PDA) Hi-media, India, and formulated culture broth that uses wheat bran wastes were routinely used for experimentation.

Preparation of fermentation medium

Wheat bran filtrate was used for the formulation of fermentation medium. The filtrate was supplemented with the following nutrient compositions: Glucose 50 g/L, Peptone water 2.5g/L, KH_2PO4 2g/L, $MgSO_4.7H_2O$ 1 g/L, $CaCl_2$ 1g/L, Yeast-extract 2.5g/L and (pH adjusted to 5.8). Three agar plugs (6mm diameter), from the *Pleurotus* culture were inoculated into each 200 ml fermentation medium in a 500 ml conical flasks. The medium were now incubated in an orbital shaker at 28°C for 7days at 150 rpm (Talaro and Talaro, 2002).

Harvest of mycelia mat from fermentation medium

After 7 days of fermentation, the medium was harvested by using cheese cloth to sieve in order to obtain the mycelia mat. The dry

weight of the mycelia mat was determined (in mg/l) using digital weighing balance while the percentage medium consumption was calculated as follows:

$$MC\ (\%) = \frac{IMV\text{-}FMV}{IMV} \times \frac{100}{1}$$

Where, % MC = % medium consumption; IMV = initial medium volume; FMV = final medium volume.

Determination of dry mycelia weight

This was carried out by drying the wet mycelia in an oven at 50°C for 24 h to remove the moisture content using pre-weighed filter paper. After drying, the dry-weight was then determined by weighing on weighing balance taking into consideration the weight of the filter paper.

Microscopy studies of wild and mutant strains

Phase contrast microscopy and scanning electron microscopy (SEM) analysis was investigated to study the morphological characteristics in both wild-type and mutants of *P. ostreatus* and *P. florida*.

Sample preparation for phase contrast microscopy

A sterile glass cover slip was inserted on surface of PDA media at an angle of about 30 or 40°C. Fungal culture was inoculated with the help of a needle at the base of the cover slip touching the media. The Petri dish was kept in an incubator at 28°C for 2-3 days for incubation. After sufficient growth of fungal mycelia, the glass cover slip was taken out and placed on glass slide stained with lactophenol-in-cotton blue. It was mounted on a phase-contrast microscope for observation of hyphae and presence of spores. Observation was done first with objective lens (10x) and after with oil immersion lens (100x).

Scanning electron microscopy (SEM)

The SEM is a microscope that uses electrons rather than light to form an image. SEM is a versatile tool for high resolution surface imaging. The SEM uses low energy secondary electrons (SEI) or high energy back scattered electrons (BEI) from the specimen surface for image formation. While SEI image provides information on 50 to 150 3Å thicknesses of the sample, BEI image reveals surface features from larger depth. The advantages of SEM over light microscopy are greater magnification, resolution and much larger depth of field. The study of the ultrastructure of biological material with SEM provides information on fundamental tissue characteristics.

Sample preparation for SEM

For SEM observations, agar plug of the fungus (0.5 cm diameter) were taken for studies and were chemically fixed in solution of 2% glutaraldehyde in 0.2 M sodium cacodylate buffer. They were then washed for 20 min in three changes of sodium cacodylate buffer 0.1 M. Post-fixation was done with 1% osmium tetraoxide in sodium cacodylate buffer at 4°C. It was then washed and dehydrated in a graded alcohol series (varying concentrations of acetone) at 4°C.

Dehydrated samples were dried in a critical point drier at its critical point, that is, 31.5°C at 1100 p.s.i with liquid carbon dioxide, mounted on brass stubs by double sided adhesive tape, and then coated with gold (35 nm thickness) in a sputter coater (JFC-1100) for analyses by SEM. The samples were viewed using scanning electron microscopy (JEOL, JSM 6360).

Molecular diversity study using random amplified polymorphic DNA (RAPD)

Isolation of DNA

The genomic DNA of the two wild-type, that is, *P. ostreatus* and *P. florida* , and their 12 fast growing mutants, making 14 fungal strains all together were extracted using the modified method of Doyle and Doyle (1990). The mycelia from *Pleurotus* spp. were harvested using a scalpel, transferred into sterilized mortar and pestle, and grinded to form a fine paste. DNA extraction buffer consisting of (1 M Tris-Cl pH 8.0; 1 M NaCl; 200 mM EDTA pH 8.0; 10% SDS; 0.1% β-mercaptoethanol) was used.

The paste was transferred into Eppendorf tube, 400 µl DNA extraction buffer was added and centrifuged at 12,000 rpm, at 4°C for 10 min. To the collected supernatant, 300 µl phenol and 300 µl chloroform: isoamylalcohol (24:1) were added and mixed gently. This was centrifuged (12000 rpm, 4°C for 10 min), and the aqueous phase was collected and 500 µl chilled isopropanol was added and incubated at -20°C overnight.

After the incubation, it was centrifuged (12000 rpm, 4°C for 10 min), and the pellet was washed with chilled 70% ethanol and centrifuged for 5 min. The dried pellet was re-suspended in 50 µl of Tris EDTA (10 mM Tris and 1 mM EDTA, pH 8.0) buffer. The isolated DNA was stored at -4°C.

RAPD PCR- Fingerprinting protocol

Random amplified polymorphism protocol uses one primer with an arbitrary sequence unlike normal PCR which uses two. Therefore, amplification in the RAPD occurs anywhere along a genome that contains two complementary sequences to the primer which are within the length limits of PCR. Initial screening was done with 10 primers (Bangalore genei).

Only four primers that gave reproducible and scorable amplifications were further used in the analysis. The reaction recipe for PCR amplification of genomic DNA was as follows: PCR buffer containing $MgCl_2$ - 2.5 µl, 10 mM dNTP mix - 2.5 µl, 10 pM primer - 2.5 µl, distilled water 14.5 µl, Taq polymerase - 1 µl, and finally added was DNA template - 2 µl. Each reaction volume (25 µl) was pippetted into Eppendorf tube and placed in thermal cycler for amplification (Applied Biosystems 9700).

The following thermal profile was applied for RAPD-PCR assay, that is, step 1 - initial denaturation at 94°C for 2 min, Step 2 - Denaturation at 95°C for 30 s, step 3 - annealing at 36°C for 45 s, step 4 - extension at 72°C for 4 - 5 min. After completion of 30 cycles, step 5 - Final extension at 72°C for 8 min.

Electrophoresis of PCR Amplicons

The amplified PCR-Product was analyzed on an agarose gel electrophoresis (1.2% w/v agarose gel). The electrophoresis was run in 1x TBE buffer: 20 mM Tris, 89 mM Boric acid, 20 mM EDTA (pH 8). The gel loading samples consists of 8 µl of the PCR amplified DNA mixed with 1.5 µl 6X gel loading buffer. A 100 bp molecular marker was used. The gel after staining with ethidium bromide was viewed under UV light and documented with a Gel Documentation system (UVP, USA).

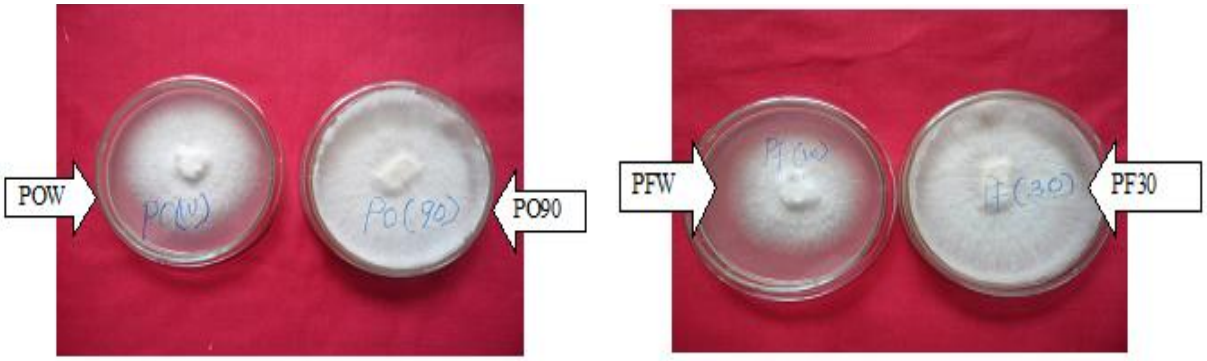

Figure 1. The radial growth rate of wild and mutant strains on potato dextrose agar plate after 5 days of inoculation. (A) *Pleurotus ostreatus* wild and mutant (POW and PO90). (B) *Pleurotus florida* wild and mutant (PFW and PF30).

Table 1. *P. ostreatus* wild type radial growth on plate when compared with the mutants.

Time (h)	Fungal strain						
	POW	PO15	PO30	PO45	PO60	PO75	PO90
24	6.67 ±1.66^{b}	6.66±2.19^{b}	10±1.15^{b}	18.33±2.02^{a}	16±2.91^{b}	15.66±2.96^{b,c,d}	13.33±1.6
48	11.6±1.66^{b,c}	15±1.73^{c}	16.33±1.85^{b,c,d}	15±3.61^{b,c}	25.75±7.55^{b,c,d}	22.33±2.33^{a,d}	18.33±4.93^{b,}
72	20±2.88^{a,c}	26.67±3.71^{a,c}	25±2.30^{a,d}	20±2.88^{a,c}	39.25±11.35^{a,d}	30±4.04^{a,c}	35.±8.66^{a,c}
96	26.66±1.6^{a,c}	29.67±5.04^{a}	33.33±4.41^{a,c}	30±1.15^{a}	47.75±16.12^{a,}	33.33±4.40^{a}	41. 66±3.33^{a,c}
120	30.33±1.45^{a}	31.67±2.03^{a}	31.66±2.84^{a}	31.66±4.33^{a}	55±22.26^{a}	38.333±6.17^{a}	48.33±4.40^{a}

*POW - *Pleurotus ostreatus* wild (POW); *PO15, PO30, PO45, PO60, PO75, PO90 are UV induced mutants respectively. Values shown by different letters in the same columns are significantly different from each other at $P < 0.05$ using Turkey Column Comparison test. Values are given as mean values ± standard deviation.

RAPD data analysis

RAPD bands were scored as present (1) or absent (0). The RAPD banding profile data obtained was entered into the NTSYS-pc version 2.02K package (Rohlf, 1998), a pairwise comparison of isolates was made, and genetic diversity parameters were determined. A dendrogram was constructed by the unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973) to group individuals into discrete clusters.

RESULTS

Selection based on the mycelia radial growth rate of wild and mutant strains of selected *Pleurotus* strains and biomass weight after submerged fermentation

After 5 days of monitoring the growth of wild type and mutants strains for both *P. ostreatus* and *P. florida* on Petri plates at the same time, there was rapid growth in the growth of mutant strains than the wild type as shown in Figure 1. The mycelia radial growth rate was also better in the mutant strains than their wild-type as shown in Tables 1 and 2. Further shown in Figure 2 is the result obtained from the performance of the *Pleurotus* strains in submerged fermentation after harvest, that indicated that mutant strains performance in terms of biomass yield were better, with (PO90) and (PF30) having the highest mycelia mat weight.

The ultrastructural studies of the fungal strains

The morphological differences in the wild and all mutant strains generated were observed by phase contrast microscope under objective lens of 100x and some of the results are shown in Figure 3. It was observed that *Pleurotus* species is a club fungi (basidiomycetes) and septated. Scanning electron microscopy was further done to see the morphological differences between the wild-type and mutant of four selected strains, that is, POW, PO90, PFW and PF30. Figure 4 showed that the hyphae network of the wild-type of *P. ostreatus* is roughened while its mutated strains are smooth. Also shown was that the mycelium strands in wild-type, stand singly, their septa was not obvious, while they have muddled up (compacted) and become bigger in mutated strains. The mutated strains under magnification showed obvious septa when compared with their wild-type. Also in *P.*

Table 2. *P. florida* wild type radial growth on plate when compared with the mutants.

Time (h)	Fungal strains						
	PFW	PF15	PF30	PF45	PF60	PF75	PF90
24	5.67 ±1.15^{b}	4±2.10^{cd}	8.33±4.04^{b}	4.66±1.15^{bc}	3.0±0.33^{bc}	7.33±0.57^{b}	6.66±0.57^{b}
48	6.67 ±1.52^{b}	6.67±2.30^{ad}	12.66±4.61^{bc}	6.66±1.15^{bc}	5±1.0^{b}	12.33±4.04^{bcd}	10±1.0^{b}
72	10±1.0^{bc}	7.33±1.52^{ac}	18.±2.0^{bc}	8.33±4.93^{b}	6.67±2.08^{b}	18.33±7.57^{ad}	11.66.±3.78^{bc}
96	4.33±1.66^{ac}	9±.2.0^{a}	23.33±13.5^{a}	14.33±1.15^{b}	8.33±1.52^{b}	20±5.0 ^{ac}	16.66±1.52^{ac}
120	20.±2.0^{a}	10.66±1.15^{a}	30±5.0^{a}	23.33±5.68^{a}	16.66±2.88^{a}	25±5.0^{a}	20±2.0^{a}

* PFW, *Pleurotus florida* wild (PFW); * PF15, PF30, PF45, PF60, PF75, Pf90 Are UV induced mutants respectively. Values showed by different letters in the same columns are significantly different from each other at P < 0.05 using Turkey Column Comparison test. Values are given as mean values ± standard deviation.

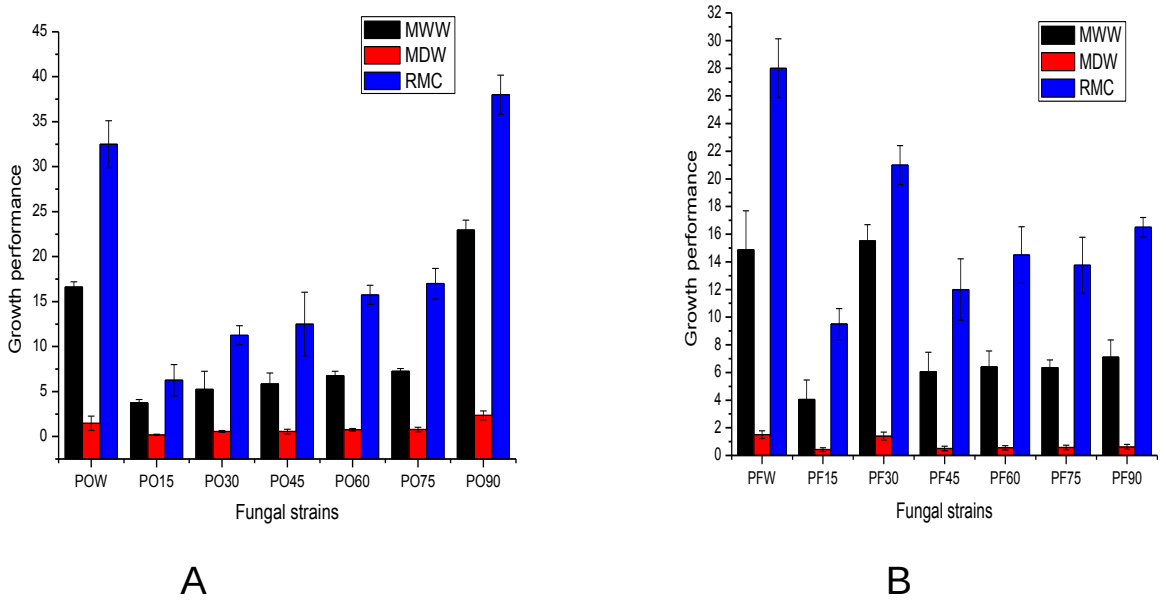

Figure 2. Comparison of mycelia weight and percentage medium consumption of *Pleurotus* fungal strains in submerged fermentation media. (A) *Pleurotus ostreatus* wild and mutant strains. (B) *Pleurotus florida* wild and mutant strains. Error bar represents standard error of means of observed values. *MWW, mycelia wet weight; *RMC, percentage medium consumption; * MDW, mycelia dry weight.

florida, the mycelia of wild-type is rough while its mutant are smooth. In addition, the mutants were well septated while the wild type was not reflected well.

RAPD fingerprinting analysis

The summary of the RAPD-PCR analysis, based on the 4 random primers revealed that Primer BG17 gave 2 polymorphic band with about 40% polymorphism among the *Pleurotus* strains, primer BG 18, 23 and 25 indicated polymorphism of 42.85% and three bands, 28.5% polymorphism and two bands, as well as 36.3% polymorphism and four polymorphic band. From analysis of the four RAPD random primers used in this study (Figure 5), based on the banding profile, also UPGMA based dendrogram was constructed (Figure 6). Also, the summary of primers used and the percentage polymorphism obtained are shown in Table 3.

DISCUSSION

In this study, mutagenesis was used to enhance biomass yield of *P.ostreatus* and *P. florida* mutants using ultraviolet irradiation. The morphological differences in the wild and mutant strains generated were observed by monitoring the mycelia growth rate on the plates, as well as determining the dry mycelia mat weight in mg/l. Also, differences in both wild and mutant strains *of P. ostreatus* and *P. florida* were shown using phase contrast microscope

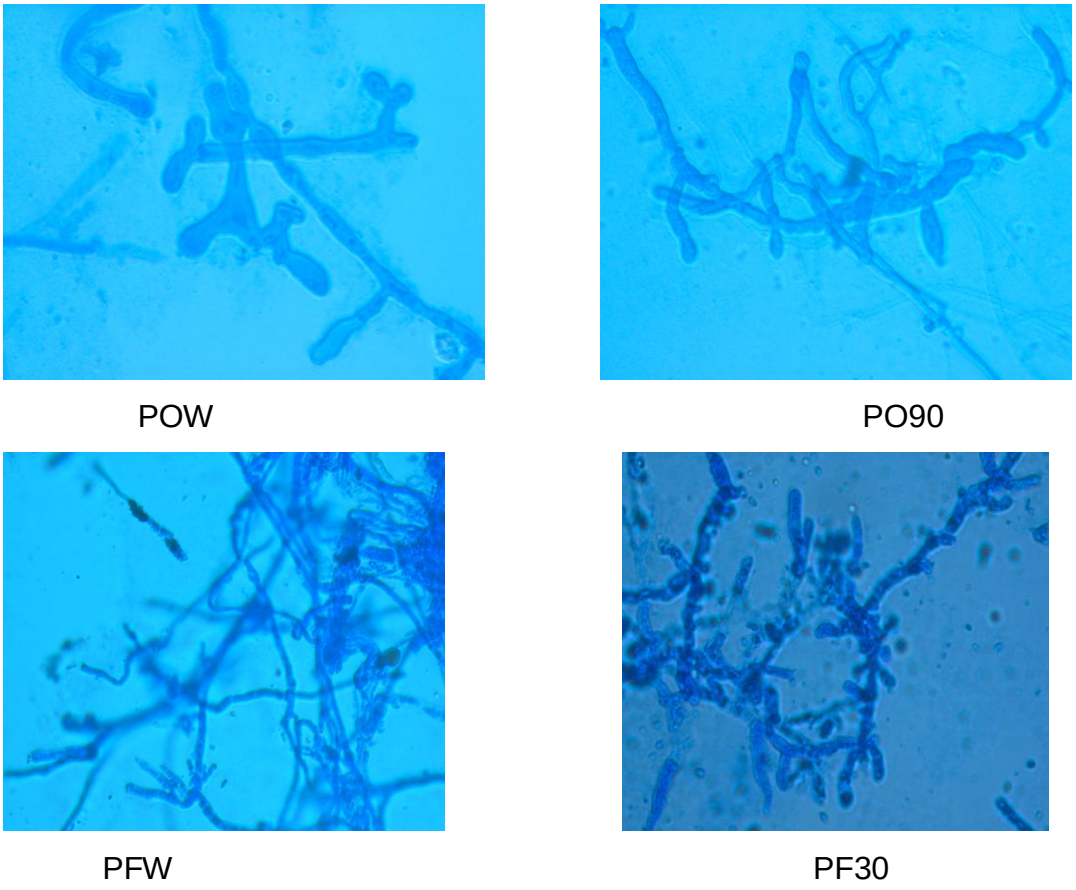

Figure 3. Microscopic observations of wild-type and mutant of *P. florida* and *P.ostreatus* under 100x objective lens of light phase microscope.

microscope and scanning electron microscopy. The result indicated that mutant strains were different from the parental wild strains. The wild strains of both *P. ostreatus* and *P. florida* mycelia were standing singly, less septate and roughened, when compared with their mutated form that were more compacted, septated and much bigger when compared with their wild type. The mycelia mat of PO90 and PF30 were significantly different from the other *Pleurotus* strains used in this study at $p < 0.05$. This result is in agreement with result of Ravishankar et al. (2006) using UV light radiation exposure to improve mycelial biomass yield and higher sporophore production. Also, the result corroborates research by Adebayo et al. (2012) in which UV mutagenesis was used to improve *P. pulmonarius* strains to increase productivity of mycelial and fruiting body yield.

From the molecular diversity studies, the RAPD images obtained from four RAPD random primers based on the presence or absence of bands, a UPGMA based den-drogram was constructed. The constructed dend-rogram consisted of two major clusters. Cluster 1 com-prised of POW, PO15, PO60, PO75, PO45, PO90, PO30 and cluster II comprised of PFW, PF30, PF45, PF75, PF15, PF60 and PF 90. The dendrogram indicated that POW, PO15, PO60 and PO75 were 100% similar. Other *Pleurotus* strains in this study ranges between 40-80% in similarity.

This indicated that truly, there was diversity in the different strains of *Pleurotus* species. The summary of the RAPD-PCR analysis, revealed that primer 17 gave 2 polymorphic band with about 40% polymorphism among the *Pleurotus* strains, primer 18, 23 and 25 indicated polymorphism of 42.85% and 3 bands, 28.5% polymorphism and 2 bands, as well as 36.3% poly-morphism as well as 4 polymorphic band. A similar result was reported by Chandra et al. (2010) using RAPD markers to discriminate eight *Pleurotus* species and also found variations in their banding patterns.

The genetic diversity result obtained in this study corroborates earlier studies reported by Stajic et al. (2005), Khan et al. (2011) and Hyeon-Su et al. (2007) using RAPD molecular marker in the diversity study of mushrooms. Stajic et al. (2005) working with 37 strains of 10 *Pleurotus* species obtained a dendrogram that grouped the strains into 6 different genetic clusters. He reported that morphological based grouping did not

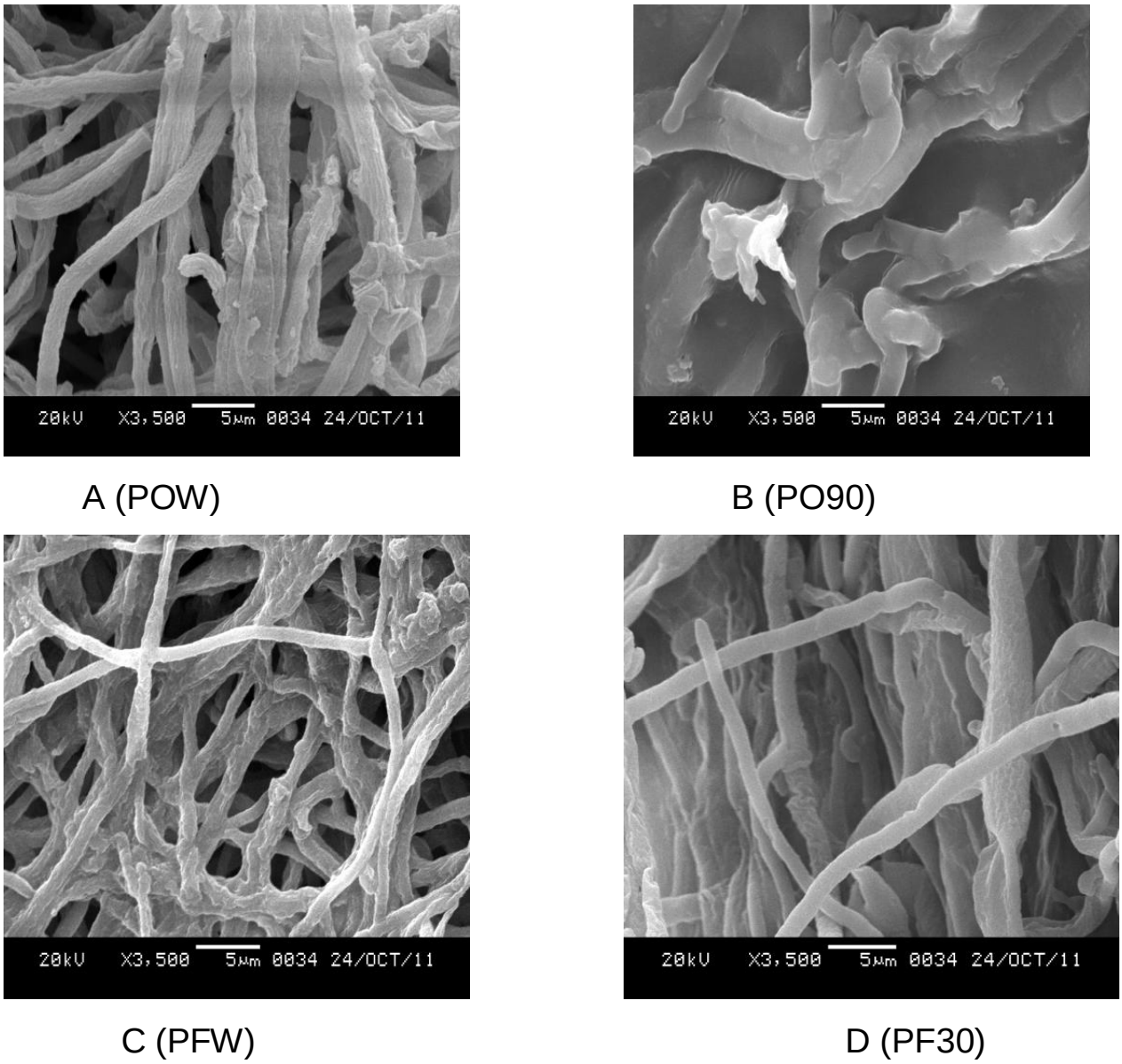

Figure 4. Scanning electron microscopy of hyphae network of wild and mutant strains of *Pleurotus ostreatus* and *Pleurotus florida*.

match genomic relationship among the species. Also, working with RAPD-PCR, Hyeon-Su et al. (2007) demonstrated that analysis of DNA amplifying pattern could be classified into five different clusters based on genetic similarity analysis and this indicated genetic diversity.

The research of Khan et al. (2011) using RAPD-PCR to analyzed genomic DNA of seven species; found out that the number of bands and banding patterns were variable depending upon the primer and type of species. The similarity matrix differentiated the species into three distinct clusters.

Studies confirmed that this techniques is a good genotypic identification analysis that is better than morphological and physiological identification, that are influenced by cultivation conditions (Iqbal et al., 2010)

Conclusion

This study confirmed that RAPD-PCR, that is a simple, cost effective technique is a good genotypic identification analysis that is better than morphological and physiological identification, that are influenced by cultivation conditions.

Mushroom growers can use RAPD analysis and morphological evaluation to characterize mushrooms in order to maintain good quality mushroom breed.

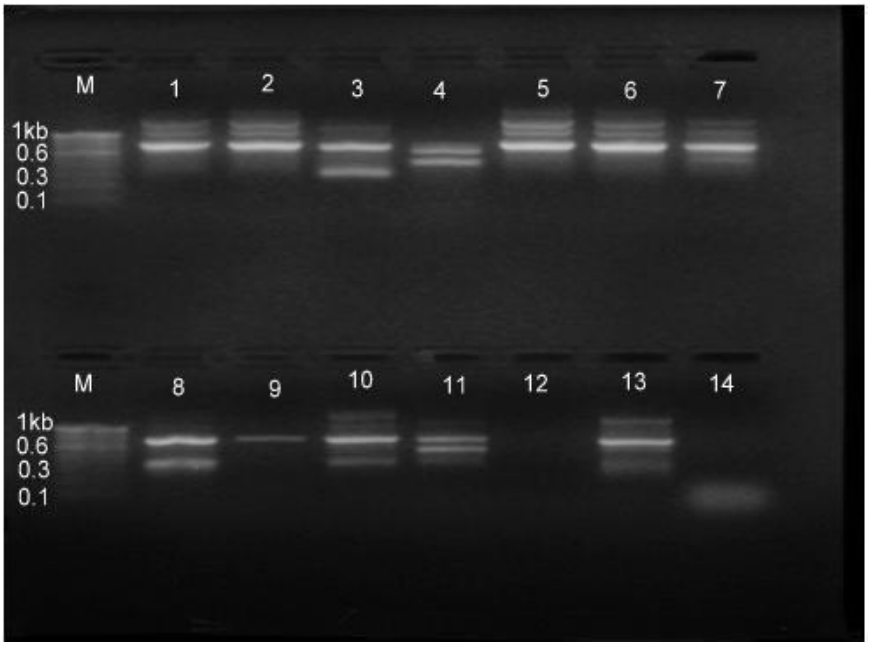

Figure 5. RAPD-PCR banding profile obtained with primer number BG17 of DNA isolated from *P. ostreatus* and *P. florida* wild and mutant strains. Lane M: 100 bp ladder, Lane 1: PfW, Lane 2: Pf15, Lane 3: Pf30, Lane 4: Pf45, Lane 5: Pf60, Lane 6: Pf75, Lane 7: Pf90, Lane 8: POW, Lane 9: PO15, Lane 10: PO30, Lane 11: PO45, Lane12: PO60, Lane 13: PO75, Lane 14: PO90.

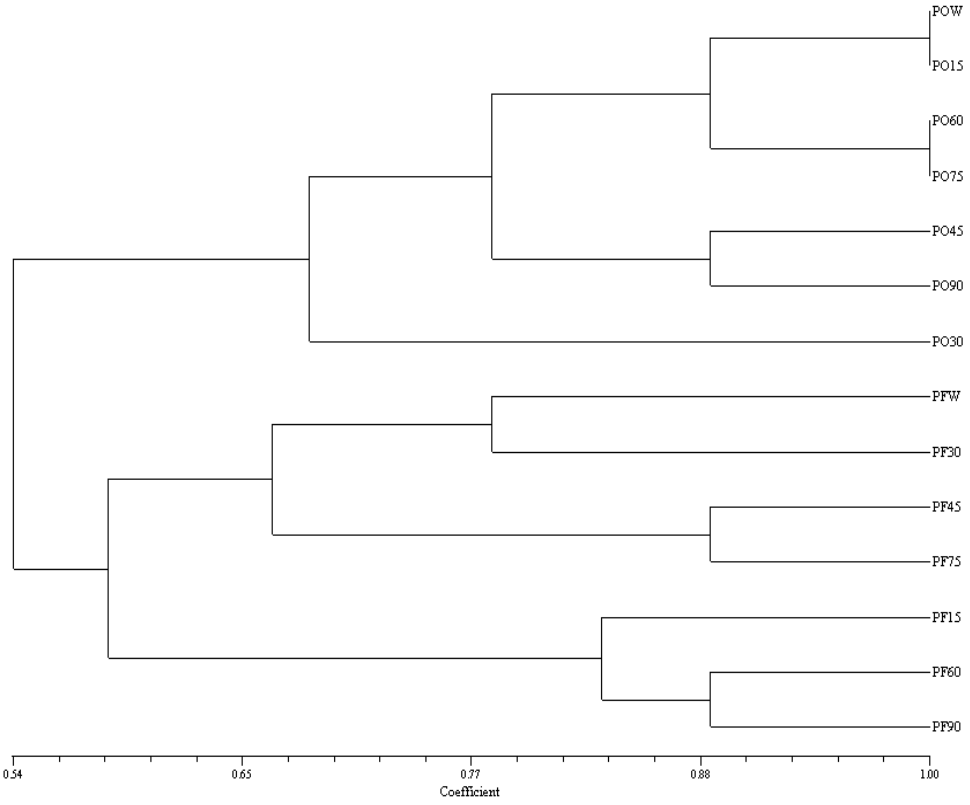

Figure 6. Dendrogram of wild and mutant strains of *Pleurotus* species constructed by combining banding profile of the four primers using neighbour-joining cluster analysis method produced from Jackard estimates using NTSYS Spc Version 2.20.

Table 3. Summary of polymorphism result of the four primers used.

Primer code	Polymorphic bands	Total bands	Percentage Polymorphism (%)	Range of band size
BG 17	2	5	40	300-1700
BG 18	3	7	42.85	300-2500
BG 23	2	7	28.5	300-2200
BG 25	4	11	36.3	500-1800
Total	11	30	147.65	
Mean/primer		7.5		
Average polymorphism			36.9	

ACKNOWLEDGEMENTS

Ms. Ola, Iyabo, is thankful to the world academy of sciences TWAS, Italy; and CSIR, India for the award of one year Postgraduate Fellowship utilized at Biotechnology division CSIR-NEIST, India, under the guide of Dr. B.G. Unni; sponsored under Network Project INDEPTH - BSC - 0111". Authority of LAUTECH, Ogbomoso, Nigeria is appreciated for granting study leave to undertake this study.

REFERENCES

Adebayo EA, Oloke JK, Bordoloi AK, Barooah M, Bora TC (2012). Improvement of *Pleurotus pulmonarius* Lau 09 through mutation for yield performance. Research Journal of Mutagenesis. pp. 1-9.

Adejoye OD, Fasidi IO (2009). Effect of cultural conditions on biomass and laccase production in submerged medium by Schizophyllum commune. (Fr.) a Nigerian edible mushroom. Electron. J. Environ. Agric. Food Chem. 8:1186-1193

Akinfemi A, Babayemi OJ, Jonathan SG (2009). Bioconversion of maize husks by white rot fungi. Revista cietifica Agricola. 9(4):972-978.

Alofe FR, Odu E A, Illoh H C (1998). Mushrooms, man and nature, edible wild mushrooms on decaying wood in Obafemi Awolowo University campus. The Nigerian Fields. 63:3-18.

Chandra S, Ghosh K, Acharya K (2010). Comparative study on the Indian cultivated *Pleurotus* species by RAPD fingerprinting. Nature Sci. 8:90-93.

Chang ST, Hayes WA (1978). The biology and cultivation of edible mushrooms. Academic press Inc. London 1:221-225.

Cohen R, Persky L, Hadar Y (2002). Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. Appl. Microbiol. Biotechnol. 58:582-594.

Declan MS, Alan DWD (2001). Differential regulation of laccase gene expression in *Pleurotus sajor-caju*. Microbiology.147:1755-1763.

Doyle, Doyle (1990). Isolation of plant DNA from fresh tissue. Focus. 12:13-15.

Guzman G (2000). Genus *Pleurotus* (Jacq.Fr) P. Kumm. (Agaricomycetideae): diversity, taxonomy problems and cultural and traditional medicinal uses. Int. J. Med. Mushrooms 2:95-123.

Hyeon-Su RO, Sung SK, Jae SR, Che-Ok J, Tae SL, Hyun-Sook L (2007). *Pleurotus eryngii*: ITS sequence analysis, RAPD fingerprinting, and physiological characteristics. Mycolog. Res. 111:710-715.

Jonathan SG, Adeoyo RO (2011). Evaluation of Ten wild Nigerian mushrooms for Amylase and Cellulase activities. Mycobiol. 39(2):103-108.

Khan S M, Nanaz A, Malik W, Javed N, Yasmin T, Rehman M, Quayyum A, Iqbal Q, Ahmad T, Khan A A (2011). Morphological and molecular characterization of oyster mushroom (*Pleurotus* spp). Afri. J. Biotechnol. 10(14):2638-2643.

Kumari D, Achal V (2008). Effects of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus* (Oyster mushroom). Life Sci. J. 5(3):73-76.

Matila P, Salo-vananen P, Kankon H, Jalava T (2002). Basic composition of mushrooms cultivated in Finland. J. Agric. Food Chem. 50(22):19-22

Ravishankar S, Pandey M, Tewari RP, Krishna V (2006). Development of sporeless / low sporing strains of *Pleurotus* through mutation. World J. Microbiol. Biotechnol. 22:1021-1025.

Rohlf FJ (1998). NTSYS-pc numerical taxonomy and multivariate analysis system, version 2.02. Exeter Publication Setauket, New York.

Smith JE, Sullivan R, Rowan NJ (2003). The role of polysaccharide derived from medicinal mushrooms in cancer treatment programs: Current perspectives (review). Int. J. Med. Mushrooms 5:217-234.

Sneath PHA, Sokal RR (1973). Numerical taxonomy: the principle and practice of numerical classification. San Francisco; Freeman. Pp. 573.

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

Stajic M, Milenkovic I, Brceksi I, Vukojevic J, Duletic Lausevic S (2002). Mycelial growth of edible and medicinal oyster mushroom [Pleurotus ostreatus (Jacq.Fr. Kumm) on selenium enriched media. Int. J. Med. Mushrooms. 4:241-244.

Talaro KP, Talaro A (2002). Foundations in Microbiology 4th ed. McGraw-Hill Higher Education, USA. Pp. 272-277.

Wasser SP (2010a). Medicinal mushroom science: History, current status, future trends and unsolved problems. Int. J. Med. Mushrooms 12(1):1-16.

Wasser SP (2010). Current status, future trends and unsolved problems in studies of Medicinal mushroom. App. Microbiol. Biotechnol. 89:1323-1332.

Yan PS, Jiang JH, Li GF, Deng CL (2004). Mating system and DNA polymorphism amplified by arbitrary primers are useful as genetic markers. World J. Microbiol. Biotechnol. 19:737-740.