Nig. J. Biotech. Vol. 35 (2018) 34-43
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v35i1.5

Molecular Characterization of *Auricularia* Spp from South-Western Nigeria using Random Amplified Polymorphic DNA (RAPD) Markers

Ekun, V.S.[1] Adenipekun, C.O.[1] Ogunkanmi, L.A.[2] Ojuederie, O.B.[3] and Igwe, D.O.[4]

[1]Department of Botany, Faculty of Science, University of Ibadan, Oyo State, Nigeria
[2] Department of Cell Biology and Genetics, University of Lagos, Lagos state, Nigeria
[3]Food Security and Safety Niche, FNAS, North-West University, Mafikeng, South Africa
[4]Department of Biotechnology, Faculty of Science, Ebonyi State University, Ebonyi State, Nigeria

Abstract

This study was conducted to investigate the genetic diversity in 48 samples of *Auricularia* species randomly collected from secondary forests in Osun (11), Oyo (10), Ondo (9), Ekiti (8), Ogun (8) and Lagos States (8) of Nigeria. Fourteen Random Amplified Polymorphic DNA (RAPD) markers were used for molecular characterization of *Auricularia* species. Phylogenetic relations were determined by cluster analysis and Polymorphic Information Content (PIC) and gene diversity determined using standard procedures. The *Auricularia* species were grouped into 6 distinct clusters based on morphological traits. The PIC value ranged from 0.5594 (OPH-15) to 0.7819 (OPB-12) and gene diversity from 0.5930 (OPH-15) to 0.7977 (OPB-12). Primer OPB-12 was the most informative for genetic diversity of *Auricularia* species. However, primer OPB-21 gave the highest number of alleles while OPB-12 showed the highest range of gene diversity and accounted for the diversity of the *Auricularia* species. The dendrogram and the principal coordinate analysis exhibited similar clustered patterns, revealing that all the tested strains could be divided into six distinct groups, each of which correlated with different geographical regions. Molecular characterisation is essential in genetic diversity studies and has proven useful in the classification of *Auricularia* spp.

Key words: *Auricularia* spp, Cluster analysis, Genetic diversity, Mushroom cultivation, Principal coordinate analysis, Polymorphic information content, RAPD markers.

Corresponding authors email-victor_segun@yahoo.com

Introduction

Mushroom is a macro-fungus with a distinctive fruiting body, that can be easily seen with the naked eye and large enough to be picked by hand, which can be either epigeous or hypogenous (Chang and Miles, 2004). Mushrooms can be identified by their distinctive umbrella-like fruiting bodies and are consumed as mushroom sauce, a special delicacy in southern Nigeria. Mushrooms are rich in protein, vitamins (B1, B2, C) and minerals in addition to other nutrients (Alofe et al., 1996; Ekpo and Aluko, 2002; Daodu, 2003). Low cholesterol levels have been reported in mushrooms which could be useful in the discovery of anti-cancer genes and pathways (Bechtel et al., 2002; Borchers et al., 2004). Wood ear mushrooms of the genus *Auricularia* have been domesticated for cultivation in different parts of the world (Kirk et al., 2017). Auricularia is the fourth most important cultivated genus of mushrooms after *Agaricus, Lentinula* and *Pleurotus* (Yan et al., 2004). *Auricularia* mushrooms have a distinct taste and morphological features when compared to other cultivated mushrooms due to their jelly-like texture and horizontally septated

basidium (Veeralakshmi et al., 2014). *Auricularia auricula* (commonly called"jelly Ear) is basidiomycetes of the family Auriculaceae.

Auricularia polytricha is an edible mushroom cultivated in tropical regions because its mycelium can grow at temperatures ranging from 10 to 40ºC (Jonathan et al., 2009). It is the most appropriate species to cultivate in tropical regions where temperatures are high (Veeralakshmi et al., 2014). *Auricularia auricula-Judae* is used as food and folk medicine in Benin, Cote D Ivoire, Ghana, Ivory Coast and Nigeria (Osemwegie et al., 2014). The conventional method of mushroom identification by morphological features such as shape and colour are not efficient in distinguishing the different types of edible and wild mushrooms. Molecular characterization is precise and much more efficient than dependence on morphological markers which are weak in terms of genetic diversity assessment. The evaluation of genetic diversity in edible mushrooms using morphological and molecular tools would promote the efficient use of genetic variations in breeding (Paterson et al., 1991) and pharmacological programmes (Du et al., 2011). Genetic diversity studies of *Auricularia polytricha* has been reported using random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) markers (Yan et al., 2004; Yu et al., 2008; Zhang et al., 2006, 2007). Nevertheless, most of the researches on *Auricuaria* sp were mainly on cultivated strains with almost no study on the genetic variation among wild strains except the study of Du et al. (2011). Due to destruction of forest habitats, mushrooms are being threatened of depletion in the forests in South Western Nigeria (Gateri et al., 2004). It is important to cultivate wild mushrooms for domestic and commercial values. There is paucity of information on the molecular characterization of *Auricularia* spp in the forests in Nigeria. Till date there is no record of characterization of *Auricularia* spp in Nigerian forests. Much emphasis has been on *Pluerotus* sp . Molecular characterization will aid accurate identification of *Auricularia* spp from other mushroom species found in the forests which will promote its utilization in Nigeria due to its numerous benefits The present study therefore investigated the genetic diversity of cultivated mushroom *Auricularia* spp in six South Western States of Nigeria using random amplified polymorphic DNA (RAPD) markers.

Materials and Methods

Forty-eight (48) mushroom samples of *Auricularia* spp. were collected from, Oyo, Osun, Ondo, Ogun, Ekiti and Lagos States in South Western Nigeria (Table 1). Three species were identified (*A. polythrica, A. auricula* and *A*. sp) based on cultural and morphological characters.

DNA extraction

A modified CTAB (Cetyltrimethylammonium Bromide) method was used for the DNA extraction (Doyle and Doyle 1987; Veeralakshmi et al. 2014). Pileus tissue of 4 days old *Auricularia* was collected and 200mg weighed for DNA extraction. The weighed *Auricularia* samples were thoroughly ground with 800ml of CTAB buffer (20 mM EDTA, 1.4 mM NaCl, 100 mM Tris-HCl pH 8.0), SDS (1.25 %), 2 % CTAB and 0.2 % β-mercaptoethanol (v/v)), incubated at 65 °C for 15 min in a water bath with occasional mixing. The ground samples were allowed to cool before adding equal volumes of phenol, chloroform and iso-amyl alcohol (25:24:1), vortexed and then centrifuged at 12000 rpm for 15 min. The supernatant was transferred to fresh sterile Eppendorf tubes without disturbing the pellets. 400µl of ice-cold isopropanol was added to the supernatant and mixed by inverting the tubes 2-5 times to precipitate the DNA and subsequently kept at -80°C for 1h. The DNA was pelleted down by centrifugation at 12000 rpm for 10 min and the dried DNA pellets obtained, were re-suspended in 100 µl of GIBCO water (Invitrogen, Carlsbad, CA, USA) and 2 µl of 10 mg/ml RNase (Qiagen Valencia, CA, USA) added to each of the samples and kept at 4°C for 30 min to get rid of RNA. The DNA quality of the extracted DNA samples was checked on 1.5% agarose gel. Following the high level of concentration of the extracted DNA samples, dilution of each DNA sample was uniformly made to 100ng/uL DNA prior to setting up PCR.

Random Amplified Polymorphic DNA (RAPD) PCR amplification

Fourteen arbitrary RAPD decamer primers obtained from Operon Technology (Almeda, CA, USA) were used for PCR amplification (Table 2). The PCR reaction protocol used is presented in Table 3

PCR cycles

The reactions were performed using Applied Biosystems thermocylcer with PCR conditions consisting of initial denaturation at 94°C for 2 min (1 cycle), denaturation at 94°C for 20 sec (40 cycles), annealing at 40°C for 2 min, extension at 72°C for 1 min and final extension at 72°C for 5 min. Amplified fragments were separated using electrophoresis method on 1.5% (w/v) agarose gel (Sigma Aldrich, USA) with 1X TBE (Tris-Boric acid-EDTA) buffer and stained with ethidium bromide (0.5mg/ml). The DNA bands were estimated using 100-bp step DNA marker (Biolabs, New England). Each sample was run twice for PCR amplification and only reproducible, relatively intense bands were scored.

Analysis of RAPD profiles

Data matrix generated from the RAPD profiles for fragments of similar molecular weight from each individual were scored as present (1) or absent (0). The data obtained from scoring the RAPD bands were used for genetic dissimilarity matrix using Jaccard's similarity coefficient (Jaccard, 1908; Ojuederie et al., 2014). Phylogenetic relations were determined by cluster analysis using unweighted pair-group method with arithmetic averages (UGPMA) with the NTSYS-pc software version 2.02 (Rohlf 1998). Multivariate grouping was done using principal coordinate analysis (PCA) with Darwin software version 5.0.0.157 while polymorphic information content (PIC) was calculated using the method of Ojuederie et al. (2014).

Table 1: Areas of collection of Auricularia samples in South Western Nigeria

S/N	Sample Identity	Location	State
1	OG1	Abeokuta	Ogun State
2	OG2	Ewekoro	Ogun State
3	OG3	Ifo	Ogun State
4	OG4	Iiebu Ode	Ogun State
5	OG5	Ikenne	Ogun State
6	OG6	Shagamu	Ogun State
7	OG7	Odeda	Ogun State
8	OG8	Odogbolu	Ogun State
9	LA1	Agege	Lagos State
10	LA2	Ojo	Lagos State
11	LA3	Apapa	Lagos State
12	LA4	Badagry	Lagos State
13	LA5	Epe	Lagos State
14	LA6	Shomolu	Lagos State
15	LA7	Ikorodu	Lagos State
16	LA8	Mushin	Lagos State
17	OY1	Akinyele	Oyo State
18	OY2	Egbeda	Oyo State
19	OY3	Ido	Oyo State
20	OY4	Iseyin	Oyo State
21	OY5	Ogbomosho North	Oyo State
22	OY6	Oluyole	Oyo State
23	OY7	Oyo	Oyo State
24	OY8	Olorunsogo	Oyo State
25	EK1	Ado Ekiti	Ekiti State
26	EK2	Ileiemeie	Ekiti State
27	EK3	Ikole	Ekiti State
28	EK4	Oye	Ekiti State
29	EK5	Irepodun/Ifelodun	Ekiti State
30	EK6	Ikere	Ekiti State
31	EK7	Iiero	Ekiti State

32	EK8	Emure	Ekiti State
33	OD1	Idanre	Ondo State
34	OD2	Ilaje	Ondo State
35	OD3	Ile Oluji/Okeigbo	Ondo State
36	OD4	Odigbo	Ondo State
37	OD5	Okitipupa	Ondo State
38	OD6	Ose	Ondo State
39	OD7	Owo	Ondo State
40	OD8	Ifedore	Ondo State
41	OS1	Boluwaduro	Osun State
42	OS2	Ejigbo	Osun State
43	OS3	Ifedayo	Osun State
44	0S4	Ifelodun	Osun State
45	OS5	Ila	Osun State
46	OS6	Irepodun	Osun State
47	OS7	Iwo	Osun State
48	OS8	Obokun	Osun State

Table 2: RAPD markers used for the amplification of DNA samples from Auricularia

S/No	RAPD primer	Primer sequence (5'-3')	Melting temperature (Tm^{o}C)
1	OPB-11	GTAGACCCGT	34
2	OPB-12	CGTTGACGCA	34
3	OPB-15	GGAGGGTGTT	32
4	OPB-20	GGACCCTTAC	34
5	OPB-21	CGACCCTTAC	34
6	OPH-3	AGACGTCCAC	34
7	OPH-5	AGTCGTCCCC	32
8	OPH-10	CCTACGTCAG	32
9	OPH-15	GCTTCGTCAG	34
10	OPT-1	GGGCCACTCA	34
11	OPT-5	GGGTTTGGCA	32
12	OPT-7	GGCAGGCTGT	34
13	OPT-10	CCTTCGGAAG	32
14	OPT-19	GATGCCAGAC	32
15	OPD-18	GAGAGCCAAC	32

PCR amplification was performed in 25µl reaction mixture using Applied Biosystems thermocylcer as presented in Table 3.

Table 3: PCR Reaction mixture (25µl)

Parameter	Value (µl)
Template DNA (100ng)	2
10X Buffer (Bioline)	2.5
2.5mM dNTPs (Bioline)	2
500U Taq DNA polymerase (Bioline)	0.2
DMSO (dimethyl sulfoxide)	1
10uM RAPD primer	1
50mM $MgCl_2$ (Bioline)	1.25
500ml DEPC-treated water	15.05

Results

Molecular characterization of Auricularia polytricha

Auricularia polytricha was characterized using 14 RAPD primers. The major allele frequency, number of alleles, genetic diversity and polymorphic information content (PIC) obtained from the 48 accessions of *Auricularia* mushrooms collected from South Western Nigeria is presented in Table 4. Allele frequency ranged from 0.3542 (OPB-15) to 0.6042 (0PH-15), gene diversity from 0.5930 (0PH-15) to 0.7977 (OPB-12) and polymorphic information content from 0.5594 (OPH-15) to 0.7819 (OPB-12). The percentage of polymorphic amplicons presented in table 4, varied from 78% (OPH-15) to 100% (OPB-11, OPB-12, OPB-15, OPB20 and OPT-10). The banding profiles of the 48 *Auricularia* genotypes using RAPD primer OPB-12 is presented in Fig. 1.

Table 4: The major allele frequency, number of alleles, genetic diversity and polymorphic information content (PIC) obtained from 48 accessions of Auricularia collected from South West Nigeria

Marker	Allele frequency	No of Alleles	Gene diversity	PIC	% polymorphism
OPB-11	0.4375	14	0.7752	0.7615	100
OPB-12	0.3958	13	0.7977	0.7819	100
OPB-15	0.3542	11	0.7891	0.7644	100
OPB-20	0.4375	14	0.776	0.7627	100
OPB-21	0.5417	16	0.6892	0.6788	94
OPH-3	0.4583	12	0.7526	0.7358	99
OPH-5	0.5625	6	0.6337	0.6005	88
OPH-10	0.4375	5	0.7188	0.6791	94
OPH-15	0.6042	5	0.5920	0.5594	78
OPT-1	0.4583	11	0.7370	0.7130	99
OPT-5	0.5417	8	0.6528	0.6195	95
OPT-7	0.4583	7	0.7196	0.6872	97
OPT-10	0.4583	16	0.7648	0.7536	100
OPT-19	0.5208	14	0.7023	0.6874	97
Mean	0.4458	13.3	0.7387	0.7176	95.8

Cluster analysis of 48 Auricularia spp from South West of Nigeria

The dendrogram constructed using jaccard's similarity coefficient summarized the interrelationships observed among the 48 *Auricularia* spp from 6 States in South Western Nigeria (Fig.2). The 48 *Auricularia* spp were grouped into six distinct clusters with a similarity coefficient of 0.646.

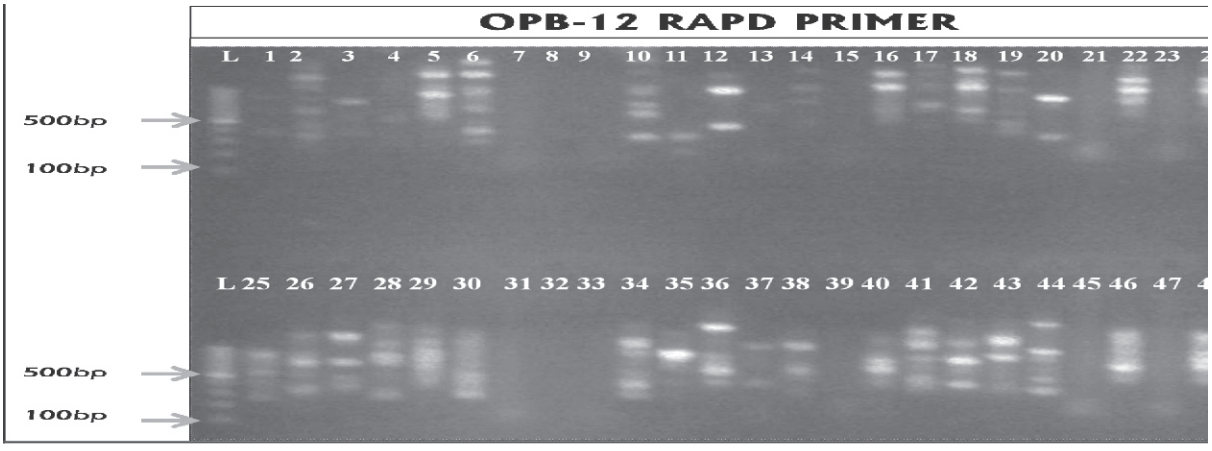

Fig 1: Banding profiles showing 48 Auricularia genotypes using RAPD primer OPB-12. L= 100 bp DNA ladder,1=OG1,2=OG2,3=OG3,4=OG4,5=OG5,6=OG6,7=OG7,8=OG8,9=LA1,10=LA2,11=LA3, 12=LA4,13=LA5,14=LA6,15=LA7,16=LA8,17=OY1,18=OY2,19=OY3,20=OY4,21=OY5,22=OY6,23=OY7, 24=OY8,25=EK1,26=EK2,27=EK3,28=EK4,29=EK5,30=EK6,31=EK7,32=EK8,33=OD1,34=OD2,35 =OD3,36=OD4,37=OD5,38=OD6,39=OD7,40=OD8,41=OS1,42=OS2,43=OS3,44=OS4,45=OS5, 46=OS6,47=OS7,48=OS8

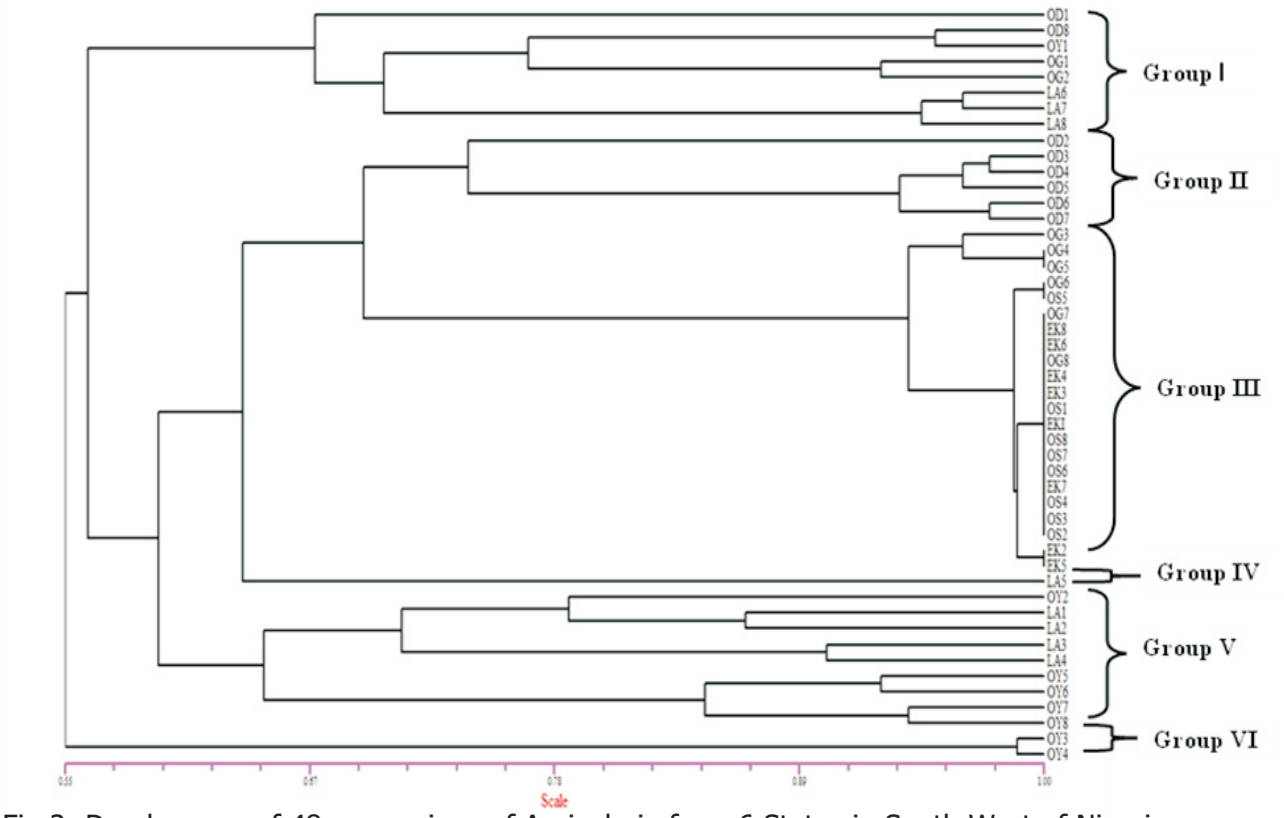

Fig 2: Dendrogram of 48 accesssions of Auricularia from 6 States in South West of Nigeria

Auricularia strains from Ekiti state EK2 and EK5 were the most closely related with 100% similarity compared to the other strains as shown on the dendrogram. The first cluster was formed by 8 genotypes (OD1, OD8, OY1, OG1, OG2, LA6, LA7, and LA8) and second cluster by 6 genotypes (OD2, OD3, OD4, OD5, OD6 and OD7). The third cluster consisted of 22 genotypes (OG3, OG4, OG5, OG6, OS5, OG7, EK8, EK6, OG8, EK4,EK3,OS1, EK1,OS8, OS7,OS6, EK7,OS4,OS3,OS2,EK2,EK5) while the fourth cluster was made of only 1 genotype (LA5) which was genetically distinct from all the other genotypes. The fifth cluster had 9 genotypes and finally the sixth cluster 6 was made up of 2 genotypes (OY3 and OY4) and was also distinct from the other genotypes.

Principal coordinate analysis of 48 Auricularia spp from South Western Nigeria

The Principal coordinate analysis (PCoA) of 48 species of Auricularia collected from the 6 States in South West of Nigeria, placed the 48 genotypes of mushroom into 6 distinct groups (Fig 3). Group1 consist of 8 locations (OD1,OD8,OY1,OG1,OG2,LA6,LA7,LA8) and Group 2 consist of 6 locations (OD2,OD3,OD4,OD5,OD6 and OD7). The Auricularia in Group 3 was found in 22 locations; OG3, OG4, OG5, OG6, OS5, OG7, EK8, EK6, OG8, EK4, EK3, OS1, EK1, OS8, OS7, OS6, EK7, OS4, OS3, OS2, EK2, EK5). Group 4 consist of only one genotype which is LA5. While genotypes in Group 5 were found in only nine locations (OY2, LA1, LA2, LA3, LA4, OY5, OY6, OY7 and OY8) and predominantly in Lagos state. The *Auricularia* in Group 6 was found in two locations of Oyo state (OY3 and OY4|) as was also observed in the dendrogram. Principal coordinate analysis (Fig 3) was consonant with the clustering data of the dendrogram in Fig 2, and confirmed the genetic distinctiveness of genotypes LA 5 in Group IV and genotypes OY3 and OY4 in Group VI

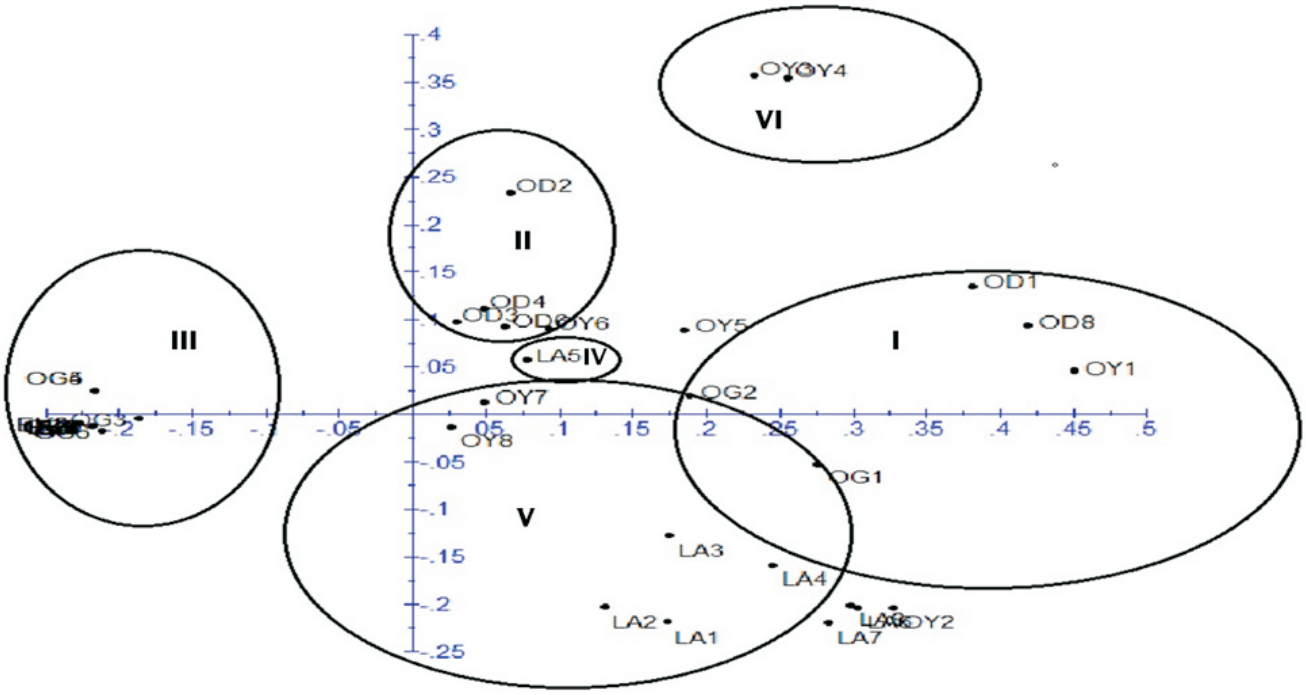

Fig 3: Principal coordinate analysis of 48 accessions of Auricularia collected from 6 States in South West Nigeria

Discussion

The genetic diversity of 48 Auricularia spp was assessed using 14 RAPD markers. RAPD markers measure the polymorphisms between the genomes of two organisms of the same species without the need for sequence information. The maximum polymorphic information content was observed in primer OPB-12 (PIC value 0.7819) followed by primer OPB-15 (PIC value 0.7644). Primer OPB-12 was the most informative primer for diversity studies in Auricularia spp evaluated. Genetic diversity studies in mushrooms have been previously reported using molecular markers especially RAPD (Ravash et al., 2009; Staniaszek et al., 2013). Other molecular markers such as rDNA sequencing, Restriction fragment length polymorphism (RFLP), and genotyping have also been used to discriminate mushroom species or strains of *Agaricus, Auricularia, Ganoderma, Lentinula, Stropharia, rugosoannulata* and *Volvariella.* All of these technologies provided data for mushroom strain identification and protection (Chandra et al., 2010). The polymorphisms revealed by the 14 decamer primers indicate that they are good and reliable for genetic diversity assessment in mushrooms and there was a high degree of diversity in the species studied. Primers OPB11, OPB12, OPB15, OPB20 and OPT10 all gave 100% polymorphism, indicating their usefulness in genetic diversity studies in *Auricularia* spp. An average polymorphism of 95.8% was obtained which was higher than the value reported by Khan et al. (2011) who conducted molecular characterization of Oyster mushroom (*Pleurotus* spp.) using 14 RAPD primers and obtained the maximum polymorphism by primers OPL3 (72.70 %) and OPL11 (70%) and the report of Ravash et al. (2009) who used RAPD markers to confirm the similarity or dissimilarity of genetic relationship of organisms. The polymorphism obtained in this study using RAPD markers was also higher (95.8%) than that obtained by Yu et al. (2008) using 13 Inter simple sequence repeat (ISSR) markers and 14 Sequence related amplified polymorphism (SRAP) markers (95.95%). In essence, the information on the difference between the genetic makeup of some macrofungi indigenous to Nigeria and the genes of their close relatives has a lot of implication on the types of bioactive compounds they can produce. But in this study, all the *Auricularia* species found in the South Western states could be grouped into six cluster regions using the result of the molecular characterization.

Group 1 consisted of 8 locations (OD1, OD8, OY1, OG1, OG2, LA6, LA7, and LA8) which must have been due to the traders moving the mushroom from one place to the other. Group 2 consisted of 6 locations (OD2, OD3, OD4, OD5, OD6 and OD7). From this group, this particular genotype was localized in Ondo State. Group 3 genotypes of mushroom were found in 22 locations (OG3, OG4, OG5, OG6, OS5, OG7, EK8, Ek6, OG8, EK4,EK3,OS1,EK1,OS8,OS7,OS6,EK7,OS4,OS3, OS2,EK2,EK5). This report shows that the genotype in Group 3 was prominent in three states and might be due to the similarity in the soil and weather conditions. In Group 4, the genotype was found in only one location (LA5). This means that it has not spread to other locations hence, was genetically distinct from the other genotypes. Auricularia in Group 5 was found in only nine locations (OY2, LA1, LA2, LA3, LA4, OY5, OY6, OY7 and OY8) predominant in Lagos and Oyo states. Finally, Group 6 was only found in two locations in Oyo state. In addition, the 6 distinct characterized *Auricularia* spp using RAPD markers showed a similarity to the morphological characterization of *Auricularia* spp under investigation. Among the main morphological factors, the change of colour of mushrooms is very common in species and depends on the environmental conditions (Vieira et al., 2013). Groups 1 and 2 *Auricularia* from morphological studies showed that they are dark brown, discoid in shape and gelatinous in texture while Groups 3 and 5 were yellowish brown, auriform in shape and leathery in texture. On the other hand, Groups 4 and 6 were brown in colour with flattened shape and rubbery in texture. Both the dendrogram and the principal coordinate analysis grouped the accessions into 6 distinct groups based on states and morphological characters. The RAPD analysis in this study has proven to be useful in discrimination, characterization and differentiation of *Auricularia* genotypes and grouped them according to similarity.

In conclusion, the study revealed the extent of genetic diversity among the genotypes of *Auricularia* spp evaluated. Genotypes EK2 and EK5 from Ekiti State were the most closely related specie with 100% similarity and genotype LA5 the most distinct from the others in all evaluations. This variability makes possible future studies in relation to their nutritional and agronomic characteristics. This study has also provided relevant information for cultivation purposes to mushroom farmers on the type and genetic variability of *Auricularia* mushroom source in South Western Nigeria and the usefulness of RAPD markers for DNA fingerprinting of *Auricularia* spp which is relevant to drug production due to its pharmacological benefits. The dendrogram and the principal coordinate analysis exhibited similar clustered patterns, revealing that all the tested strains could be divided into six distinct groups, each of which correlated with different geographical regions. This is the first report on molecular studies of *Auricularia* spp in Nigeria. The genetic diversity obtained using the RAPD molecular markers can be associated to key morphological features observed in the evaluated Auricularia mushrooms from South Western Nigeria

.

References

Alofe, F. V., Odeyemi, A. O. and Oke, O. L. (1996). Three wild mushrooms from Nigeria: their proximate and mineral composition. Plant food Human Nutr. 49: 63 - 67.

Bechtel, A., Butuzova, L. and Turchanina, O. (2002). Thermochemical and geochemical characteristics of sulphur coals. Fuel. Proc. Technol .**77 –** 78:45 – 52. https://doi.org/10.1007/s00216-011-5171-4

Borchers, A. T., Keen, C. L. and Gershwin, M. E. (2004). Mushrooms, tumors and immunity: An update. Exp. Biol. Medici .229(5):393 - 406. https://doi.org/10.1177/153537020422900507

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

Chang, S. T. and Miles, P. G. (2004). Mushrooms: Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact, CRC Press, Inc. Florida, p. 451.

Daodu, O.O. (2003). Effect of different lime concentrations on the cultivation of an edible fungus (*Pleurotus sajor caju*; Oyster mushroom). M.Sc. Thesis, University of Ibadan, Nigeria.

Doyle, J. J. and Doyle. J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bullet. 19:11 - 15.

Du, P., Cui, B. and Dai, K. (2011). Genetic diversity of wild *Auricularia polytricha* in Yunnan Province of South-western China revealed by sequence-related amplified polymorphism (SRAP) analysis. J. Med. Pl. Res .5(8):1374 - 1381.

Ekpo, E. N. and Aluko, A. P. (2002). Cultivation strategies and nutritive values of edible mushrooms (Lentinus tuber-regium) as a component of sustainable livelihood. Proc 28th Ann Conf Forestry Assoc Nigeria.

Jaccard, P. (1908). Nouvelles resecherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44:223 – 270.

Jonathan, S. G., Bawo, D. D. S., Adejoye, D. O. and Briyai, O. F. (2009). Studies on biomass production in *Auricularia polytricha* collected from Wilberforce Island, Bayelsa State, Nigeria. Am. J. Appl. Sci. 6:182 - 186.

Khan, S. M., Nawaz, A., Malik, W., Javed, N., Yasmin, T., Rehman, M. U., et al. (2011). Morphological and molecular characterization of Oyster mushroom (*Pleurotus* spp.). Afr. J. Biotechnol. 10(14):2638 - 2643. http://dx.doi.org/10.5897/AJB10.499

Kirk, P. M., Cannon, P. F, David, J. C, Stalpers, J. A. (eds) (2001). Ainsworth & Bisby's dictionary of the fungi. 9th edition. CABI Publishing, Wallingford.

Ojuederie, O. B., Balogun, M. O., Fawole, I., Igwe, D. O. and Olowolafe, M. O. (2014). Assessment of the Genetic Diversity of African yam bean (*Sphenostylis stenocarpa* Hochst ex. A. Rich Harms) Accessions using Amplified Fragment Length Polymorphism (AFLP) Markers. Afr. J. Biotechnol. 13(18):1850 - 1858. DOI.10.5897/AJB2014.13734

Paterson, A. H., Tanksley, S. D. and Sorrells, M. E. (1991). DNA markers in plant improvement. Adv. Agron . 46:39 - 90. DOI: 10.1016/S0065-2113(08)605 - 787

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

Rohlf, F. J. (2000). NTSYS-pc numerical taxonomy and multivariate system (v.2.1). User guide. Exeter Software, Setauket, New York USA 31pp.

Royse, D. J. and Lee C. S. (2013). Mushrooms Their Consumption, Production and Culture Development, Interdisciplinary Sci. Rev. 5:4:324-332, DOI: 10.1179/isr.1980.5.4.324 .

Staniaszek, M., Marczewski, W., Szudyga, K., Maszkiewicz, J., Czaplicki, A. and Qian, G. (2002). Genetic relationship between Polish and Chinese strains of the mushroom *Agaricus bisporus* (Lange) Sing., determined by the RAPD method. J. Appl. Genet. 43: 43 - 47.

Veeralakshmi, S., Ahila, Devi, P., Prakasam, V. and Thiribhuvanamala, G. (2014). Molecular characterization and standardization of cultivation for wood ear mushroom [*Auricularia polytricha* (Mont.)] Sacc. Int. J. Biotechnol. Res. 2(5): 060 - 064.

Vieira, F. R., Pereira, D. M ,. Nogueira de Andrade, M. C. and Minhoni, M. T. (2013). Molecular characterization of *Pleurotus ostreatus* commercial strains by Random Amplified Polymorphic DNA (RAPD). Afr. J. Agric. Res. 8(24)3146-3150. DOI: 10.5897/AJAR2013.7160

Yan, P. S., Luo, X. C. and Zhou, Q. (2004). RAPD molecular differentiation of the cultivated strains of the jelly mushrooms, *Auricularia auricular* and *A. polytricha*. World J. Microbiol. Biotechnol. 20:795 - 799. Yan, P. S., Luo, X. C. & Zhou, Q. World Journal of Microbiology and Biotechnology (2004) 20: 795. Doi.org/10.1007/s11274-004-5840-y

Yu, M. Y., Ma, B., Luo, X., Zheng, L. Y., Xu, X. Y. and Yang, Z. R. (2008). Molecular diversity of *Auricularia polytricha* revealed by inter-simple sequence repeat and sequence related amplified polymorphism markers. Curr. Microbiol. 56:240 - 245.

Zhang, D., Zheng, Y. L., Gao, J. W., Wu, W. and Chen, H. (2006). Studies on germplasm resources of *Auricularia polytricha* by inter-simple sequence repeat (ISSR). .J Mountain. Sci. 24:142-148.14.

\Zhang, D., Zheng, Y. L., Wang, B., Wu, W. and Chen, H. (2007). Studies on germplasm resources of *Auricularia polytricha* by random amplified polymorphic DNA. Biotechnol. Bull 18:117 - 123