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Cultivation and Sporophore Formation of an Indigenous Nigerian Isolate of *Auricularia polytricha* on Sawdust and Palm Waste Substrates

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ABSTRACT

Auricularia species is a jelly- like edible fungus usually with mild flavour. It is also known as wood ear mushroom because of its ear-shaped morphology. Nigeria is blessed with many species of these edible mushrooms; however, not much research effort has been given to the cultivation of *Auricularia* species. Wild species of *Auricularia* were collected from different locations within southern Nigeria in their natural habitats. Pure cultures of these species were obtained and identified using both phenotypic and molecular tools protocols. The spawn of *Auricularia* species was produced using paddy and sorghum grains in Federal Institute of Industrial Research, Oshodi (FIIRO). The rates of ramification of the spawn on the two substrates were monitored where Sorghum grains ramified on the 28days (4weeks) earlier than the paddy grain which was on the 35 days (5 weeks). Cultivation of the mushroom species on a mixture of sawdust, palm waste and rice bran at varying ratios was conducted. Full mycelia ramification of substrates occurred at 28 days for palm waste (100%) and palm waste: sawdust: rice bran (80:10:10), compared to 35 days for sawdust-based substrates ($P<0.05$). The highest biological efficiency (22.8%) was obtained with Palm waste: Sawdust: Rice bran (80:10:10) while 3.5% was obtained with (100%) palm wastes substrates. Molecular identification confirmed the isolate as *Auricularia polytricha* with accession number KP942817. In conclusion, Nigerian strain of *Auricularia* species was successfully cultivated on agro wastes.

Keywords: *Auricularia polytricha*, agro waste, molecular identification.

1.0 Introduction

Auricularia specie (wood ear mushrooms) are members of the Kingdom Fungi, Division Basidiomycota, Class Agaricomycetes, Order Auriculariales, and Family Auriculariacae, (Kirk, 2015). They are jelly-like edible fungi with mild flavour and are widely distributed across temperate tropical and subtropical reions (Kirk *et al.,* 2008; Singh, 2008; Mukerji and Manoharachary, 2010). Common species include *Auricularia auricula-judae* and *Auricularia polytricha*, which typically grow on decaying hardwood and may occur singly or in clusters (Kuo, 2002; Chang, 2006).

Morphologically, Auricularia species are characterize by ear-shaped, gelatinous fruiting bodies that are elastic when fresh but become hard and brittle upon drying (Sterry and Hughes, 2009;Kumari *et al*., 2013). Their colour ranges from reddish-brown to darker shades with age, often exhibiting folded or wrinkled surfaces (Hanson *et al*., 2003).

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Due to their nutritional and functional properties, *Auriculria* species have gained importance as edible mushrooms. They contain relatively high protein levels (16-25%), comparable to legume (Liu *et al*., 2009), and are ranked among the most cultivated mushrooms globally after *Agaricus, Lentinus, and Pleurotus* (Yan *et al.,* 2009). However, in some regions, including parts of Africa, they are still largely harvested from the wild rather than cultivated commercially (Akpaja *et al.,* 2003; Elizabeth *et al.,*2016).

Therefore, this study aimed to: (1). Identify an indigenous *Auricularia* isolate using molecular methods. (2). Evaluate spawn production on sorghum vs. paddy grains. (3).Determine the optimal agro-waste substrate mixture for mycelia ramification and biological efficiency.

2.0 Materials and Method

Sources of Materials

All the reagents and chemicals used in this study were of analytical grade, and procured from accredited agents of the manufacturers. All the media and reagents were prepared according to the manufacturer's instructions.

Strain Collection and Isolation

Fresh fruiting bodies of *Auricularia* species were collected from seven (7) different locations in Nigeria from their natural habitat. Ten (10) grams of fresh samples coded with EW001, EW002, EW003, EW004, EW005, EW006 and EW007 were washed with sterile distilled water and surface sterilized using 10% ethanol for 2 seconds. Samples were inoculated on Potato Dextrose Agar (PDA) supplemented with streptomycin and incubated at 25°C. Pure cultures were maintained on PDA slants at 4°C.

Spawn Production

Spawn was produced using sorghum and paddy grains Federal Institute of Industrial Research, Oshodi (FIIRO) Biotechnology laboratory. Grains were boiled, drained, supplemented with 1% dry weight $CaCO_3$, sterilized, and inoculated with actively growing mycelia. All experiments were conducted in triplicates (n=3).

Substrate Preparation

Sawdust (SW) and Palm waste (PW) substrates were prepared and supplemented with rice bran (RB) at varying ratios (100% SW, 50% SW:50% RB, 70% SW:30% RB, 30% SW:70% RB and 100% RB). Moisture content was adjusted to 60-65% with digital moisture meter for instant reading (Onyango *et al.,* 2011). Substrates were sterilized at 121 ^{o}C for 2hrs 30 mins and inoculated with spawn produced from Biotechnology Department of Federal Institute of Industrial Research Oshodi, Lagos Nigeria, then incubated at 25±2°C until full colonization.

Biological Efficiency

Biological Efficiency (BE) was calculated as;

BE (%) = (Total Fresh mushroom weight /Total Dry substrate weight) × 100

Where: Total fresh mushroom weight is the total weight of all mushroom harvested over all flushes.

Total dry substrate weight is substrate weight before adding water.

Molecular Identification

DNA extraction and ITS amplification

The genomic DNA from the isolates of *Auricularia polytricha* was extracted from the fungal hyphae by a modified DNA extraction protocol (Elizabeth *et al.,* 2016; Adebayo *et al.,* 2012). Internal Transcribed Spacer (ITS) region of the rRNA gene was amplified, using universal ITS1-F and ITS4-R primers. PCR reaction consisted of PCR IX buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl), 200 µM of each deoxinucleotide, 1.5 mM $MgCl_2$ 50 pmols of each oligonucleotide, 2.5U Taq DNA polymerase (sigma, India) and 50 mg DNA, in final volume of 50µL. The PCR was performed on a Thermal cycler (Perkin-Elmer, Norwalk, USA) by the following parameters: initial denaturation at 94°c for 3 min, then 35 cycles of 94°c for 1 min, 55°c for 1 min, extension for 72°c for 1 min and final extension for 72°c for 10 mins; then final holds at

4°c. The amplified products were screened on 1.2% agarose gel.

Sequencing and phylogenetic analysis

The PCR products were purified using BigDye Terminator cycle sequencing kit, (Applied Biosystems, USA). The sequencing was carried out using ABI Prism 3100 genetic analyser with a 36 well comb and a 48- cm WTR (well-to-read) gel (Applied Biosystems, USA). Electrophoresis was set at 2400 V for 10-11 hours. The high quality ITS sequences read using CondonCodeAligner (CondonCode, USA). Sequences confirmed in the 3' and 5' were taken into consideration for assembly to generate the consensus sequence of ITS. The identification of the phylogenetic neighbours and the calculation of pairwise ITS gene sequence similarities were achieved using the EzTaxon server (Chun, *et al.,* 2007). Also, sequence similarity tool BLAST was employed against ITS database of N CBI to retrieve gene sequences from related taxa and was edited in BioEdit (htt://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequences selected from similarity search (cut-off 95% identity) including the query sequence were aligned using ClustalX (Larkin *et al.,* 2007) which was further used for phylogeny reconstruction. Phylogenetic analysis was performed using MEGA6.0. a distance matrix method viz distance options according to the Kimura two-parameter model (Kimura, 1980), including clustering by neighboring–joining (Saitou and Nei, 1987), maximum likelihood methods (Tamura,*et al.,* 2011) were used. The robustness of the phylogeny in each case was estimated by Boostrap analysis based on 1000 replications. The nucleotide sequences of the partial ITS gene of the species were deposited in GenBank database of NCBI and were assigned accession numbers.

3.0 Results

Spawn Production

Sorghum grain supported faster mycelia growth (28 days) compared to paddy grain (35 days). Independent samples t-test showed a significant difference ($P< 0.05$).

Pure cultures of the isolated species were successfully used to produce spawn of the isolated *Auricularia* species on both paddy and sorghum grains in (Federal Institute of Industrial Research, Oshodi) FIIRO laboratory. Rate of mycelia ramification was faster on sorghum grain than paddy grain with full ramification achieved after 28 and 35 days for sorghum and paddy grains respectively. There was a significant difference $P<0.05$ between rate of ramification in sorghum and paddy grains with values of means of 59.444 and 41.944 respectively (Table 1).

Table 1: Mycelia Ramification on Grain Substrates

WEEKS	SORGHUM	PADDY GRAIN
1	15.67±4.04	12.33±2.52
2	50.00±5.00	36.67±2.89
3	78.67±2.89	61.67±2.89
4	100.00±0.00	76.67±2.89
5	-	100.00±0.00

Mycelia Growth on Substrates

Palm waste based substrates showed faster colonization compared to sawdust substrates. Isolated species were cultivated on different ratios of sawdust substrate supplemented with rice bran and Palm wastes for sporophores formation. Full ramification of substrate was achieved after 35 days from the date of inoculation for sawdust (100%), sawdust: rice bran (70%:30%), (30%:70%), (50%:50%). Palm waste (100%), Palm waste (80:10:10) ramified faster than substrates with sawdust on the 28 days of inoculation. Palm waste (100%) ramified fastest than substrates with and without sawdust substrate on the 4th week of inoculation followed by sawdust (100%) on 5th week then sawdust with different ratios of supplements (Rice bran). At 0.00% the substrate has fully ramified. Produced sporophores were big and wide with brownish colour. Palm wastes substrate supplemented with sawdust and rice bran had the highest biological efficiency (22.8%), followed by sawdust (100%) with 12.9.0%. Palm waste (100%) had the lowest biological efficiencies of 3.5% (Table 3).

Table 2: Mycelia Ramification (%) Over time

WEEK	SAWDUST 100%	SW:RB 70:30	SW:RB 30:70	SW:RB 50:50	PW:SW:RB 80:10:10	PW 100%
WK1	10.01±0.14	10.00±0.73	10.00±0.11	12.00±1.22	36.00±1.11^{d}	25.00±0.84
WK2	30.00±0.42^{d}	15.00±0.71	15.01±0.84	25.00±0.84	65.001.38d	50.00±0.63^{d}
WK3	45.00±0.68^{d}	32.00±1.22^{d}	32.00±1.22^{d}	41.20±0.97	90.00±0.63^{d}	70.00±0.84
WK4	65.00±0.36^{d}	50.00±0.24^{d}	51.20±1.24^{d}	60.00±1.38	100.00±0.63^{d}	100.00±2.02
WK5	100.00±0.76^{d}	70.00±0.58^{d}	60.00± 1.1^{d}	85.00±0.84^{d}	0.00±0.00	0.00±0.00
WK6	0.00±0.00	100.00±1.62^{d}	85.00± 1.7^{d}	100.0±0.6^{d}	0.00±0.00	0.00±0.00
WK7	0.00±0.00	0.00±0.00	100.00±0.25^{d}	0.00±0.00	0.00±0.00	0.00±0.00

Values represent mean ± SD(n=3). The superscript letter (d = $p < 0.0001$) shows the level of significance

Table 3: Biological efficiency of *Auricularia polytricha*

Mushroom yield

Substrate	1st flush (g)	2nd flush (g)	3rd flush (g)	BE (%)
Sawdust (100)	11.9	9.0	2.0	12.9
SW:RB 70:30	10.0	8,5	2.5	12.3
SW:RB 30:70	9.5	7.3	1.5	10.7
SW:RB 50:50	12.0	8.0	2.0	12.9
PW:SW:RB 80:10:10	20.0	12.0	4.0	22.8
PW 100%	5.0	-	1.0	3.5

NCBI accession numbers of *Auricularia polytricha* that were used for cultivation in the study are as follows: EW001 (Accession number KP942817), EW002 ((Accession number KP942818), EW003 (Accession number KP942819), EW004 (Accession number KP942820), EW005 (Accession number KP942816), EW006 (Accession number KP942814) and EW007 (Accession number KP942815). The first tree result revealed that all sequences shared a common ancestral origin with three subcluster, the subcluster 1 shows the three *Auricularia polytricha* closely related and they share same cluster with 100 boostrap values. Three *Auricularia polytricha* of query sequences with accession numbers KP942817, KP942818 and KP942820 as shown in Figure 1. The results shows that the isolate with query sequence KP942820 *Auricularia polytricha* and HM448468 *Auricularia polytricha* shares a common ancestral origin to the closest branch with the second query sequence KP942818 *Auricularia polytricha* and every other branches as they diverged going down the tree and query sequence KP942817 is the outgroup due to random mutations that occurred over time in the distance species during evolutionary process. It can be deduced that the tested species used in this experiment have high genetic relationship compared to that of the NCBI database.

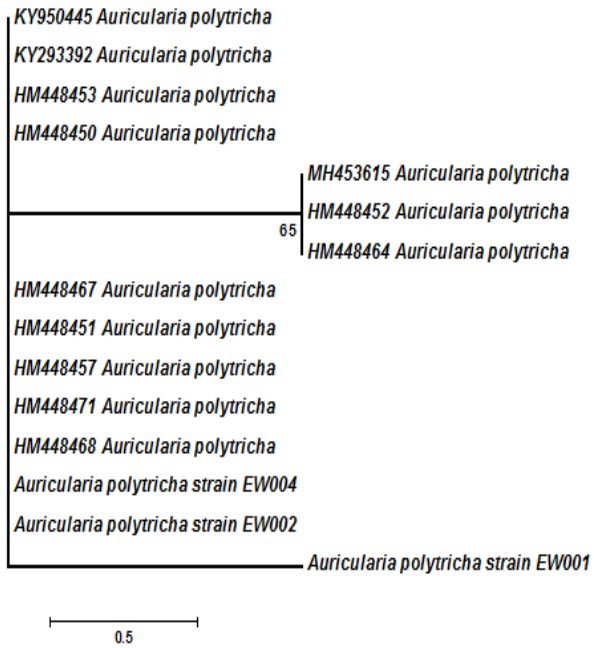

Figure 1: Phylogenetic tree of *Auricularia polytricha* isolated from the wild and compared with other species of *Auricularia polytricha* in NCBI database.

Discussion

The increasing demand for a continuous supply of high quality mushrooms has necessitated the search or suitable substrates for the cultivation and domesticated of edible mushroom species.

Auricularia species are edible mushrooms with significant nutritional and medicinal potential. In the present study, Seven (7) *Auricularia* species were isolated from the wild, and their domestication, including spawn production and substrate suitability, was evaluated.

Spawn production is a critical step in mushroom cultivation. In this study, paddy and sorghum grains were assessed as substrates for spawn production of *Auricularia* species. The results showed that the rate of mycelia ramification was faster in sorghum grain compared to paddy grains, with full colonization achieved within 28 and 35 days respectively. This indicates that sorghum grain is a more suitable substrate for spawn production. Similar variations in mycelial growth rates among mushroom species have been reported by Adebayo *et al.,*2012, a 14 day ramification period for production of spawn of *Pleurotus* spp; while Samota and Sharma, (2011) observed a 49 days running period for full ramification of spawn Shiitake mushroom. It is therefore apparent from these results that the rate of mycelia running during spawn production varies from one mushroom species to the other.

The suitability of different substrate formulations for the cultivation of *Auricularia polytricha* was also evaluated using sawdust supplemented with rice bran and palm waste in varying proportions. Full mycelia colonization was achieved after 35 days of incubation across most sawdust based substrates. This finding contrasts with Irawati *et al.,* (2012), who reported a shorter colonization period of 14 days when *A.polytricha* was cultivated on palm waste. However, early mycelia ramification was observed as early as the fourth day of inoculation in substrates containing palm waste combine with sawdust and rice bran. This observation is consistent with Abdulrazak *et al.,* (2012), who recorded enhanced mycelia growth in substrates supplemented with oil residues and spent grain.

Biological efficiency varied among the substrate formulations, with the combination of palm waste, sawdust and rice bran (80: 10: 10) recording the highest value (22.8%), which was significantly higher than that of sawdust alone (12.9%). This result is in agreement with abdulrazak *et al.,* (2012), who reported higher biological efficiency in palm based substrate mixtures compared to sawdust alone. The improved performance may be attributed to better nutrient availability and substrates composition.

Primodial formation occurred within 10 days of exposure across all substrates. This differs from the finding Onyango *et al.,*(2012), who reported primodial emergence 8 – 13 days depending on substrate type which support efficient enzymatic degradation and nutrient release.

Furthermore, the sporophores produced in this study were relatively large and well developed. This observation agrees with Stamets (2000), who reported that vigorous mycelia growth often results in the production of large and healthy fruiting bodies.

Conclusion

This study demonstrates that *Auricularia polytricha* can be successfully cultivated using locally available agro-wastes such as palm waste and sawdust substrates in Nigeria. Further research is needed to optimize yields, validate biological efficiency with proper weight-based measurements, and assess the economic viability of small-scale production.

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