ASSESSMENT OF CROSS COMPATIBILITY IN THREE STRAINS OF *PLEUROTUS SPECIES* AND YIELD ATTRIBUTES OF THE SURVIVING PROGENIES

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ABSTRACT

Despite the upsurge in mushroom consumption in Nigeria, breeding of new strains for improved quality, early maturity and biological efficiency is a new concept. This study investigated cross-compatibility among three mushroom parental strains (*Pleurotus ostreatus, P. tuber-regium* and *Lentinus edodes*) and assessed yield attributes in parents and the progenies of compatible strains – (*P. ostreatus* and *L. edodes)*. The crosses were carried out at the Department of Biology, Kogi State University, Anyigba, Kogi State, Nigeria using the dual culture technique. Among the twenty-seven combinations, only five (5) hybridized successfully. Crosses between *P. tuber-regium* and the other two strains (*P. ostreatus* and *L. edodes)* failed to yield viable progenies while the cross between *P. ostreatus* (po) and *L. edodes* (le) yielded five (5) new strains. Data collected on morphological and yield attributes of the compatible parents and the new strains revealed that three (3) strains (po1 x le1, po3 x le2 and po3 x le3) produced white fluffy colony growth morphology which represented the characters of *P. ostreatus* while the remaining two strains (po2 x le1 and po2 x le3) had milky, appressed morphology, representing the attributes of *L. edodes*. The strain from cross combination po3 x le3 completed spawn run in 37 days which was the fastest ($\rho < 0.05$) among the strains and also exhibited superiority ($\rho < 0.05$ or $\rho < 0.01$) for all other attributes including fresh yield and biological efficiency over other strains and the two parents. Two other strains *viz*. po2 x le3 and po3 x le2 also exhibited significantly ($\rho < 0.05$) higher yield and biological efficiency than either of the parental strains. These new strains can be exploited for commercial production of improved mushrooms in Nigeria.

Key words: Cross Compatibility, *Pleurotus*, Dual Culture, Yield, Biology efficiency

INTRODUCTION

Mushrooms are fleshy, spore-bearing reproductive structure of fungi which are important as human food due to its nutritional and medicinal properties (Bllal *et al*., 2010). They are universally recognized as sources of high quality protein that can be produced with greater biological efficiency than animal protein (Wang *et al*., 2001). Although they are commonly found in the wild, mushrooms can also be grown under household condition in a sustainable way to create a source of livelihood and improved nutrition. Since it requires low resources and can be grown all year round, mushroom cultivation is the most efficient and economically viable biotechnology for the conversion of lignocellulose waste materials into high quality protein food and fertilizers (Jaradat, 2010).

Edible mushrooms particularly *Pleurotus species* are known to be among the largest of fungi which grow on different agricultural wastes producing fruiting bodies and in the process, convert the substrates into digestible protein-rich substance which is suitable for animal feed, soil conditioners and fertilizer. The genus Pleurotus (oyster mushroom) comprises some of the most popular edible mushrooms because of their favourable organoleptic and medicinal properties as well as vigorous growth and undemanding cultivation conditions (Gregori *et al*., 2007). The growth period of *P. ostreatus* is usually shorter than that of other edible mushrooms (Sánchez, 2010).

Mushroom breeding has great potential as a means of growing a highly nutritious food with excellent taste from substrates that are in abundance and grossly underutilized. A corollary is that the development of new and improved strains of mushroom with better quality, early maturity and biological efficiency will meet the increasing demand of the people and become a major landmark for the establishment of commercial production of new mushroom cultivars with novel and improved traits (Larraya *et al.,* 2003). This is also expected to provide the mushroom industry with options for solving food problems and increase the efficiency of mushroom production.

Several approaches such as protoplast culture, spore germination, ultra violet (UV) light and ethyl methyl sulfonate (as mutagens) among others have been used to breed new strains of mushroom (Aswini *et al.,* 2014; Sharma and Sharma, 2014). However, the protoplast fusion technique has

proven to be the most feasible method for inter-specific and inter-generic hybridization for strain improvement among edible mushrooms. For example, Djajanegara and Masduki (2010) as well as Parani and Eyini (2010) reported success of protoplast fusion in obtaining improved F_1 hybrids from the cross of *P. eous* and *P. flabellatus*. Luk and Chiu (2005) also utilized the protoplast fusion approach to generate interspecific hybrids between *Ganoderma lucidum* and *G. tsugae* to enhance high and low temperature tolerance mushroom strain. In their own study, Chakraborty and Sikdar (2010) used intergeneric protoplast fusion between *Calocybe indica* and *P. florida* to generate hybrid strains with improved qualitative and quantitative sporophore of the milky mushroom. This technique has also been used to derive improved strains with high biological efficiency ranging between 119 and 153% from a cross between *P. eryngii* and *L. edodes* neohaplonts, apart from a wide variety of morphologies including different pileus size, colours and shapes, large, thick and fleshy stipe, resulting in their suitability as strains for a commercial exploitation (Ramirez *et al*., 2011). Other successful studies in protoplast fusion include those which produced strains of *P. ostreatus* that are adapted to warm environment (Gaitán-Hernández and Salmones, 2008) hybrid mushrooms which produces anti-thrombin agents obtained from a cross between *Laetiporus sulphureus* and *H. marmoreus* (Okamura *et al*., 2000) and another hybrid obtained from fusion of protoplast of *P. florida* and *Volvariella volvacea* with immunoactive polysaccharide (Patra *et al*., 2011).

Although mushroom is a popular delicacy in many communities especially in the southern Nigeria where it is exploited for food and medicinal purposes (Okhoya *et al*., 2010), there is yet no breeding programme designed for the development of high yielding and nutritious mushrooms strains in the country. In the study reported herein, crosses were effected among three species of *mushroom –P. ostreatus, P. tuber-regium* and *Lentinus edodes* with the objectives to (i) determine the level of compatibility between the three species and (ii) evaluate the new strains for their yield traits and productivity.

MATERIALS AND METHODS

The experiment was carried out at the Department of Biological Science Research Laboratory, Kogi State University, Anyingba, Nigeria. Three species of mushroom *Pleurotus tuber regium, P. ostreatus* and *Lentinus edodes* were used for this study. The spores of *P. ostreatus* mushroom was obtained from the National Biotechnology Research Development Agency, Ogbomosho, Nigeria while those of *P. tuber-regium* and *L. edodes* were obtained from the Department of Biology, Kogi State University, Anyingba.

Test for Compatibility

Spore cultures each derived from three Mushroom strains were selected at random and compatibility test was carried out with a combination of cultures. The dual culture technique was used to make crosses among the homokaryotic cultures. This was done by insertion of a thriving mycelia of cultures of single spores of each of the strains about 1 cm away from each other in an agar made of potato dextrose contained in a petri dish of about 100 mm (Badalyan *et al*., 2002). The combination was in three replicates, set up as a completely randomized design (CRD). After the union of the homokaryons a mycelia sample was removed and placed in to fresh agar medium and advanced into heterokaryotic mycelia. Upon observation of a network of dikaryotic hyphae clamp, at the growing edges of the strains interacting, the crosses were deemed successful. The hybridized strains were kept under room temperature. Based on the growth performance of the hybrids, only cross combinations between *P. ostreatus* and *L. edodes* successfully hybridized while crosses between *P. ostreatus* and *P. tuber-regium* as well as between *P. tuber regium* and *L. edodes* were not compatible (Table 1). Consequently, only the successfully hybridized strains and their parents were in subsequent use for further investigation.

Production of Spawns

The strains of the parents and the F_1 hybrids considered successful were established on sorghum (*Sorghum bicolor*) spawn substrate mixed with Calcium Carbonate at one percent (1%). The combination was optimized adjusted to a moisture of 60% according to the protocol described by Yang (1986) and then put in bottles. The bottles were autoclaved and thereafter were inoculated with the strains and nurtured in the dark at room temperature while enclosed in polyethylene bags (Plate 1). The treatments were in three replicates and arranged in a similar manner as for the

compatibility test. The development of the mycelia was visually detected and the number of days required for the spawn substrate to be colonized entirely by each of the strain was referred to as the percentage of medium colonization.

Fruiting and Harvesting:

The growth medium contained saw dust (78%), wheat bran (20%), CaCO3 (2%) with adequate water. The mixture was placed in various polypropylene bags and their mouths were covered by insertion of water absorbing cotton with the aid of plastic rings. The bags were placed in an autoclave at 121°C at 15-20 *psi* pressure and thereafter allowed to cool. The bags were inoculated after sterilization with the spawns of the parental and successful hybrid strains at the rate of 5% per bag according to the dry weight of substrates and then incubation for spawn run was carried out under complete darkness at the room temperature. The experiment was also laid out in CRD with three replicates. The bags were placed in the cropping room for initiation of fruiting after the mycelia reached the bottom and watered daily at cropping. During the period of the study, mature fruit bodies were harvested three times.

Data Collection

Data were collected on days to completion of spawn run, number of fruit bodies developed in bags inoculated with each of the strain, total weight of fruiting bodies (flush) using a weighing balance and productivity (%) designated as the biological efficiency of the strain. This was calculated as the total weight of fruit bodies/ Total weight of substrate spawn x 100 (Musakhail *et al*., 2011).

Data Analysis

Data collected were analyzed using GENSTAT and means were separated by Least Significant Difference according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Cross Compatibility

The cross combinations of *P. ostreatus* x *P. tuber-regium* and *P. tuber-regium* x *L. edodes* was unable to produce any successful hybrid while the cross between *P. ostreatus and L. edodes* successfully hybridized yielding five (5) new strains (Table 1). The incompatibility reaction between *P. tuber*

regium and the other two parental strains had earlier been reported by Vilgalys *et al*., (1996) who suggested that *P. tuber regium* should be expunged from among the other Pleurotus species. The inability of *P. tuber regium* to produce successful hybrids with *P. ostreatus* and *L. edodes* may as a result of pairing difficulties encountered during meiosis suggesting that *P. tuber regium* is genetically diverse from the other two species. Hyphal fusion between two homokaryotic mycelia, leading to the development of a heterokaryotic mycelium which is fertile; This development is accompanied by nuclear division and formation of septum in the connection of clamps is a proof of compatibility between the strains. The hybridization between *P. ostreatus and L. edodes* is further exemplified by two (2) types of clamp connections – a single clamp connection as well as double clamp connections (Plate 2).

Colony Morphology on PDA Medium

The morphology and colony growth characteristics of the parents and the five (5) new strains derived from the *P. ostreatus* and *L. edodes* crosses are presented in Table 2. Three (3) of the new strains (po1 x le1, po2 x le1 and po3 x le3) exhibited the morphological attributes and growth colony of *P. ostreatus* (white, smooth texture and long stipe) while the remaining two strains exhibited attributes of *L. edodes* (milky, rough texture and short stipe). However, with respect to colony characteristics, the results revealed that three (3) of the new strains (po1 x le1, po3 x le2 and po3 x le3) exhibited the colour and mycelia appearance of *P. ostreatus* (white and fluffy) while the remaining two (2) strains (po2 x le1 and po2 x le3) although had the milky colour of *L. edodes* expressed new mycelia which differed from those of the parents.

One of the new strains (cross po3 x le3) completed spawn run faster than the others including the two parental strains (Table 3) which was also accompanied by production of a large basidiocarp (Plate 3). One of the parents (*P. ostreatus*) also completed the spawn run faster than the remaining new four (4) strains and the second parent (*L. edodes*) which was the latest in completing spawn run. The variance in number of days it took to complete the spawn run among the mushroom genotypes was seven (7) days and the time required from the day of spawning to first harvest was also the least in the strains resulting from cross combinations po3 x le3 and po2 x le1 with a range of four (4) days between these strains and *L. edodes*.

However, there was no consistent trend among the strains for these two attributes since strains po2 x le1 and po3 x le2 which were the slowest in spawn run attained first fruit harvest earlier than the other strains except po3 x le3 with which were similar for this trait. Although previous studies have shown that spawn run and time taken to fruit body harvest is dependent on the growth medium, log size, spawn quality, strain, moisture, temperature and other variables (Roysne and Bahler, 1989; Ramirez *et al.,* 2011), this study did not vary the substrate. Therefore, the observed differences among the strains for these characters are likely due to recombination among the genes in the parental strains which manifested in the F_1 hybrids. Additionally, the first flush of yield was at its peak in all the genotypes while the final flush was the least (Figure 1). Two of the new strains (po3 x le3 and po2 x le3) in that order, had higher flushes of yield than either parent or the remaining strains at each period of measurement. All the new strains yielded higher than the low yielding parent (*L. edodes*) by between 24.57 g and 3.78 g (first flush), 7.8 g and 1.2 g (second flush) as well as 6.63 g and 1.02 g (third flush) representing 25.18-4.72, 24.35-4.72 and 24.38-4.72 percent (%) superiority of the new strains respectively over *L. edodes*. This suggests that *P. ostreatus* carries the allele for the expression of these traits in the dominant form.

The cumulative flush yields for each genotype also revealed a significantly higher flush yield in the two new strains (po3 x le3 and po2 x le3). Although, number of fruit bodies remained unaffected in the newly developed strains, there was an increase in weight of the fruit bodies recorded for the new strains compared to weight of fruiting bodies from either parent except for cross combinations po2 x le1 and po1 x le1 with relatively lower yields than *P. ostreatus*. In other words, the three new strains are superior to either parent in productivity and quality of fruit bodies, which agrees with earlier observations of Bak *et al.,* (1996) who reported higher productivity and quality of fruit bodies in 60 strains of shiitake raised from four parent strains compared to their parents.

Biological efficiency is an indication of the strain's capacity to convert the medium into a form that is more usable (Kumara and Edirimanna, 2009). The range in biological efficiency among the mushroom genotypes was approximately 20% with cross combination po3 x le3 which recorded the highest fresh yield also having the highest biological efficiency of 80.0. However, the values reported for biological efficiency in this study are lower

than those recorded by Gaitán-Hernández and Salmones (2008) who reported a range of 74.4 to 320 in *L. edodes* strains raised on wheat straw inoculated with three types of spawn but higher than values reported by Musakhail *et al.,* (2011) for *P. ostreatus* raised on gram powder amendment substrate. Three of the new strains exhibited superiority for biological efficiency over the parents suggesting recombination of alleles which although present in the parental strains were probably covered by their respective dominant alleles. Consequently, the three new strains may be referred to as transgressive segregants having exhibited values that are higher than those of the better parent. Furthermore, all the new strains also had higher biological efficiency ranging from 20.5 and 3.5% than *L. edodes* which is the least efficient.

Buah *et al*. (2010) as well as Masarirambi *et al*. (2011) noted that spawn running is an indication of how fast the substrate is utilized by the mycelia. Kumara and Edirimanna (2009) attributed maximum utilization of substrate to the kind of strain grown on a specific medium such that some strains may perform well in one medium while others do not. In this study, three of the new strains derived from *P. ostreatus* and *L. edodes* crosses apart from days to completion of spawn running, exhibited superiority for all the attributes measured suggesting that they exhibited better utilization of the substrate used.

In conclusion, the three new strains of mushroom which exhibited superiority for yield and quality attributes offer opportunities for commercialized production of nutritious and quality mushrooms at a much lower cost compared to earlier approaches. The study also confirmed earlier report of Vilgalys *et al*. (1996) who suggested the removal of *P. tuber-regium* from other species of Pleurotus.

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Table 1: Series of Compatibility reactions between three strains of *Pleurotus*.

Crosses	Compatibility Reaction		
P. ostreatus x *P. tuber regium*	1	2	3
1	-	-	-
2	-	-	-
3	-	-	-
P. ostreatus and L. edodes	1	2	3
1	+	-	-
2	+	-	+
3	-	+	+
P. tuber regium x *L. edodes*	1	2	3
1	-	-	-
2	-	-	-
3	-	-	-

Note: + = Compatible cross; - = Incompatible cross.

Table 2: Morphology of basidiocarp and colony growth characteristics (on Potato Dextrose Agar medium) of parents and new mushroom strains derived from *Pleurotous ostreatus* x *Lentinus edodes* cross.

Strains		Morphological Attributes			Colony Characteristics	
Parents		Colour	Texture	Stipe		
P. ostreatus		White	Smooth	Long	White	Fluffy
L. edodes		Milky	Rough	Short	Milky	Appressed
P. tuber regium		White	Rough	Long	----	---
New strains[+]	Cross code					
Strain 1	Po1 x Le1	White	Smooth	Long	White	Fluffy
Strain 2	Po2 x Le1	White	Smooth	Long	Milky	Appressed
Strain 3	Po2 x Le3	Milky	Rough	Short	Milky	Appressed
Strain 4	Po3 x Le2	Milky	Rough	Short	White	Fluffy
Strain 5	Po3 x Le3	White	Smooth	Long	White	Fluffy

+; Successful hybridization between *P. ostreatus* x *L. edodes.*

Table 3: Days to completion of spawn run, pinhead formation and average number of fruiting bodies to parents and strains derived from *P. ostreatus* x *L. edodes.*

Strains		Days to completion of spawn running (no)	Time to first harvest days)	Number of fruiting bodies (no)	Fresh yield (g)	Biological Efficiency (%)
Parents						
P. ostreatus		38	9	12	130	65.0
L. edodes		45	12	10	121	60.5
New strains[+]	Cross code					
Strain 1	Po1 x Le1	41	11	11	129	64.5
Strain 2	Po2 x Le1	42	8	6	127	63.5
Strain 3	Po2 x Le3	40	10	10	150	75.0
Strain 4	Po3 x Le2	42	9	10	140	70.0
Strain 5	Po3 x Le3	37	8	12	160	80.0
Mean		40.71	9.57	10.14	136.7	68.36
Range		7	4	6	39	19.5
LSD α0.05		3.50	3.25	8.61	0.69	0.38

+; Successful hybridization between *P. ostreatus* x *L. edodes.*

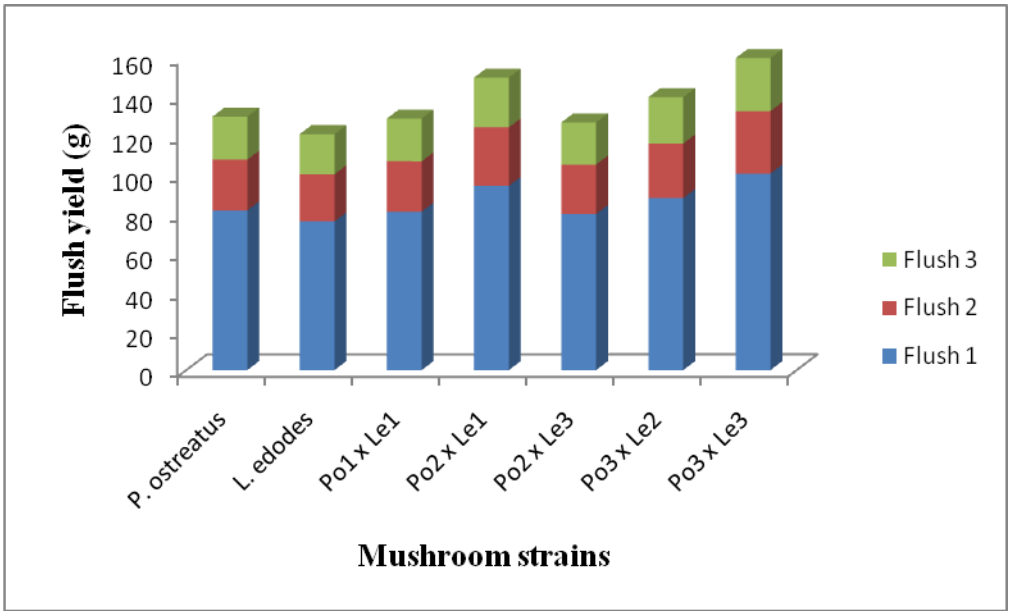

Figure 1: Flush yields at different periods of harvest in parents and new strains of mushroom derived from P. ostetreatus x L. edodes cross.

Plate 1: Development and fruiting of mushrooms in polyethylene bags inside growth room at room temperature.

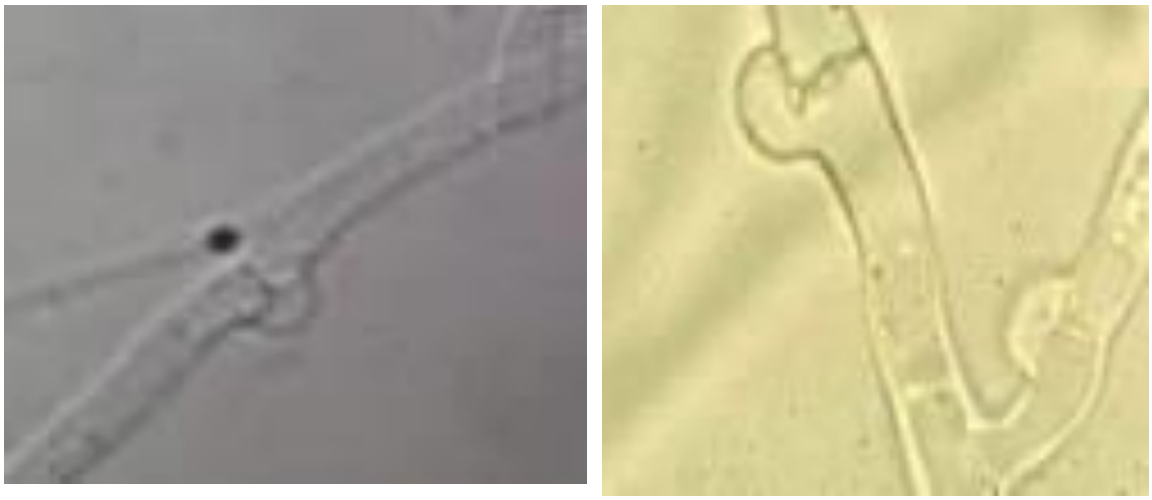

Plate 2: Clamp connection between P. osreatus and L. edodes at one point (left) and two points (right) under 100 x magnifications.

Plate 3: Basidiocarp developed from strain 5 of P. osreatus x L. edodes cross.

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