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Full Length Research Paper

Optimization of sub-merged culture conditions for biomass production in *Pleurotus florida* (mont.) Singer, a Nigerian edible fungus

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Submerged culture conditions such as physical factors, chemical compounds and inoculum sizes were optimized for biomass production in *Pleurotus florida*, a Nigerian edible mushroom. This fungus produced the greatest biomass at pH of 6.5 and 30°C. Among the monosaccharides, glucose stimulated the best biomass production (186.7 mg/30 cm^3) followed in order by fructose mannose, and sorbose (P ≤ 0.05). In the series of complex sugars and sugar alcohols, mannitol supported the highest biomass yield with mycelial dry weight of 130.0 mg/30 cm^3, followed closely by dextrin with 123.3 mg/30 cm^3 while the least value was obtained with arabitol. Tryptophan and alanine enhanced moderate vegetative growth with values, which are not statistically different from each other (P ≤ 0.05). Leucine was found to be the least amino acid for biomass production in this fungus while yeast extract was the best complex nitrogen sources. The maximum biomass yield (330.0 mg/100 cm$^{3)}$ was obtained when 7.0 cm^3 of *P. florida* inoculum was inoculated into a submerged medium while the least biomass was obtained with 0.5 cm^3 of the inoculum.

Key words: *Pleurotus florida*, submerged culture, physical factors, biomass.

INTRODUCTION

Pleurotus florida (Mont.) Singer, is a typical edible species of oyster mushroom in Nigeria. This fungus (referred to as a variant of *P. oestratus* by some authors) is widely distributed in West Africa and other tropical countries (Alofe et al., 1998; Jonathan, 2002). It belongs to phylum basidiomycetes, order agaricales and family tricholomataceae (Alexopolous et al., 1996; Zoberi, 1972). In South-western Nigeria, this mushroom grows naturally during the early and late raining season (April – June and September – November). The fruit bodies of *P. florida* develop in large number as a group or turfs on fallen tree logs and at the base of dead wood or poles. The pileus ranges from 1.5 to 8.5 cm in diameter while the stipe is very short and ranges from 0.1-0.5 cm in length. This mushroom, which possesses a weak odour of edible mushroom lack annulus and spore print, is creamy white.

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. It is unfortunate that up till now, little work has been carried out on the growth requirements of indigenous mushrooms from Nigeria to facilitate their cultivation on a commercial scale. In this study, attempt was made to investigate the mycelial biomass production in *P. florida* with the aim of providing useful information that could aid its cultivation biotechnology in Nigeria.

MATERIALS AND METHODS

Source of microorganism

The sporophores of *Pleurotus florida* were collected from the decaying wood of *Terminalia superba* at the Zoological Gardens, University of Ibadan, Ibadan, Nigeria. The fruitbodies of this mushroom were tissue cultured to obtain mycelia culture, which

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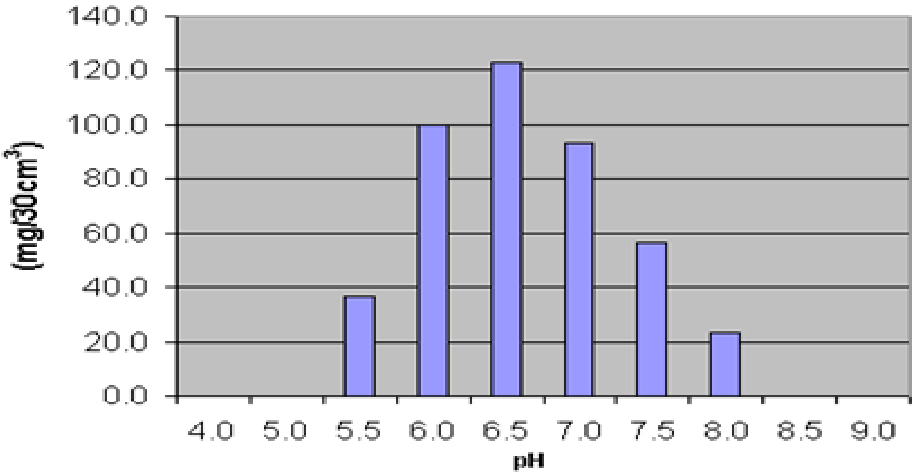

Figure 1. Effect of pH on biomass production in *Pleurotus florida*.

was maintained on the yeast extract enriched (0.5 %) potato dextrose agar (Jonathan and Fasidi, 2003).

Temperature and pH

The temperature and pH requirements of *P. florida* were determined by mycelial dry weight method using a chemically defined medium (Jonathan, 2002). For temperature, the liquid medium was dispensed into 150 ml conical flasks (30 ml per flask) and the mouth was sealed with aluminium foil. The flasks were autoclaved at 1.02 kg cm^{-2} at 121°C for 15 min. After cooling, each conical flask was inoculated with mycelia disc (7.0 mm diameter) from 6-day-old culture of *P. florida* and incubated at 30±2°C for 10 days. Each treatment was replicated three times. The mycelial were harvested using the method of Jonathan (2002). For pH, the same basal medium was employed but the medium was adjusted to pH values of 4.0–9.0. 30 ml of each treatment was dispensed into 150 ml conical flask and replicated three times. They were autoclaved, inoculated and incubated as described in the temperature experiment.

Monosaccharides, oligosaccharides and complex sugars

The basal medium used was that described by Jin-zhong et al., 2003. This chemically defined medium has the following composition peptone (Difco), 2.0 g, yeast extract (Difco), 2.0 g; KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.6 g and 1000 cm^3 of de-ionized water. The medium was supplemented separately with 20.0 g per litre of each carbon compound (monosaccharides, oligosaccharides and sugar alcohols). For polysaccharides, the medium was supplemented with 10.0 g per litre. The control medium lacked all these carbon compounds. Streptomycin sulphate (0.05 g) was added to the basal medium after sterilization to suppress bacterial growth. After homogenization, pH was adjusted to 6.5 and 30 ml of each treatment was added into 150 ml conical flask in triplicates. These were autoclaved at 1.02 kg cm^{-2} at 121°C for 15 min. After cooling, each bottle was inoculated with 7.0 mm (diameter) disc of 6 day old mycelium and incubated at 30±2°C for 10 days. The mycelial were harvested, oven dried at 80°C for 10 h and weighed. Assessment of mycelial density was carried out using the procedure described by Kadiri and Fasidi (1994).

Amino acids, inorganic and organic nitrogen sources

The basal medium used was made up of glucose, 10.0 g; Nacl, 0.1 g; $CaCl_2$, 0.1 g; KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g; thiamine hydrochloride 0.5 mg. The medium was supplemented separately with amino acids and inorganic nitrogen sources at the rate of 1.0 g per litre. Complex nitrogen sources were supplemented at concentration of 2.0 g per litre. The liquid without any nitrogen source served as the control. 30 ml of the liquid medium was dispensed into the conical flask and treated as described in the carbon experiment.

Effect of Inoculum sizes of *Pleurotus florida* on biomass yield

The basal medium used consisted of tryptophan (2.0 g), yeast extract (2.0 g), thiamine hydrochloride (500 µg), glucose (20.0 g), KH_2P0_4 (1.0 g), $MgSO_4.7H_2O$ (0.5 g) and 10.0 cm^3 of micronutrients. Demineralised water was added to make up to 1000 cm^3. The component of the micronutrient solution in 1000 cm^3 of de-mineralized water are H_3BO_4 (1.4 g) $ZnSo_4$ (0.22 g), $CuSO_4.H_2O$ (0.08 g), $NH_46MO_7.4H_2O$ (0.05 g) and 0.01 g of $FeSo_4$ (Jonathan, 2002). An aqueous homogenate of *P. florida* mycelia previously sub cultured on yeast extract enriched (YEPDA) plates grown to the size of 60-80 mm in diameter was prepared using Warring blender with 50.0 cm^3 autoclaved de-mineralized water per YEPDA plate. Different concentration of *P. florida* homogenate, (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 cm^3) were then inoculated into 250 ml conical flasks containing 100 cm^3 of the chemically defined basal medium. Incubation was carried out for 10 days at 30±2°C after which the mycelial were harvested, oven dried at 80°C for 2 days and weighed.

Statistical analysis

Mean values of biomass yield obtained from these studies were analyzed by one way Analysis of variance (ANOVA) and tests of significance difference were determined by Duncan's multiple range test (Snedecor and Cochran, 1987).

RESULTS AND DISCUSSION

Figure 1 shows that *P. florida* could produce different

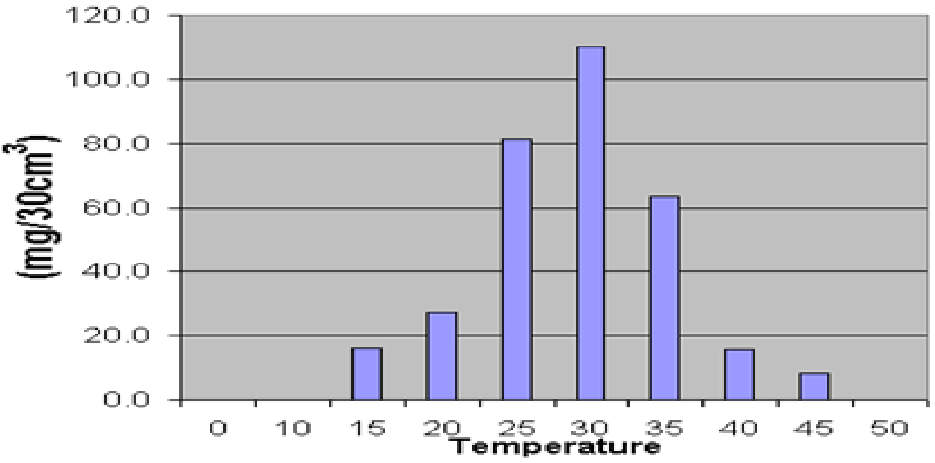

Figure 2. Effect of temperature on biomass production in *Pleurotus florida*.

Table 1. Influence of monosaccharides and oligosaccharide on biomass production in *P. florida.*

Sugars	Biomass yield (mg/30 cm^3)	Mycelial density
Monosaccharides		
L(+)arabinose	63.3±3.3ef	+3
D(-)fructose	153.3±4.5b	+7
D(+)galactose	75.0±2.9de	+4
D(+)glucose	186.7±5.8a	+7
D(+)mannose	126.7±2.7c	+6
L(-)sorbose	91.6±1.8d	+5
D(-)rhamnose	55.0±4.4def	+2
Oligosaccharides		
Cellobiose	48.3±3.8def	+2
Lactose	50.0±2.9def	+2
Maltose	101.7±3.3d	+6
D(+)raffinose	85.0±4.2de	+4
Sucrose	46.7±3.2def	+2
Control (Basal medium)	28.3±5.4e	+1

Data represented above are means of three treatments ±SE at 5% level of probability. Values followed by different letters are significantly different by DMRT ($P \leq 0.05$).

quantities of biomass within pH range of 5.5 and 8.0. Although there was a moderate mycelial yield between 6.0 and 7.5, the best biomass production was recorded at pH 6.5. This fungus produced optima biomass yield in slightly acidic medium. There were no biomass production at pH 4.0, 5.0, 8.5 and 9.0. This result agrees favourably with that of Jonathan et al. (2004) and Chandra and Purkayastha (1977) on *Volvariella esculenta* and *Agaricus campestris* respectively. Fungi are generally known to carry out their metabolic activities at acidic pH (Griffin, 1994; Hudson, 1992; Fasidi, 1996).

The result shown on Figure 2 indicates that *P. florida* produced biomass between temperatures of 10 and 45°C. The best mycelial yield (110.0 mg/30 cm) was obtained at 30°C. At 25°C, biomass yield of 81.6 mg/30 cm^3 was recorded. After 30°C, there was a gradual decline in biomass production as the temperature increases. At 50°C, no mycelial growth was observed. This result is similar to that obtained by (Gbolagade et al., 2006) for *Lentinus subnudus*. Temperatures between 25 and 30°C (room temperature) have been described as supportive for optima biomass and exopolysaccharide production in basidiomycetes (Maziero et al., 1999).

All the tested simple sugars (mono and oligosaccharides) supported different levels of biomass production (Table 1). The most supportive sugars were found among the monosaccharides. Aldohexose (glucose) stimulated

Table 2. Influence of complex sugars and sugar alcohols on biomass yield of *P. florida*.

Complex Sugars	Biomass Yield (mg/30 cm^3)	Mycelial density
Polysaccharides		
Soluble starch	61.7±2.7cd	+3
Cellulose	65.0±3.8c	+3
Dextrin	123.3±4.4a	+5
Malt extract	96.7±3.5b	+4
Sugar alcohol		
Arabitol	40.0±2.8	+2
Mannitol	130.0±3.7	+5
Myo-inositol	56.7±1.9	+3
Sorbitol	71.6±3.3	+4
Control (Basal medium)	28.3±3.7	+1

Data represented above are means of three treatments ±SE at 5% level of Probability. Values followed by different letters are significantly different by DMRT ($P \leq 0.05$).

Table 3. Influence of amino acids on biomass production in *Pleurotus florida*

Amino acids	Biomass yield (mg/30 cm^3)	Mycelial density
L-aspartic acid	50.0±2.9d	+3
L-asparagine	63.3±3.3cd	+3
D-alanine	151.6±5.3a	+7
L-glutamic acid	80.0±4.4c	+5
L-glutamine	106.6±3.8b	+6
D-cysteine	45.0±6.7d	+2
DL-methionine	100.0±2.5b	+6
L-tryptophan	160.0±4.8a	+7
DL-phenyl-alanine	73.3±3.3c	+4
DL-leucine	40.0±1.8d	+2
L-lysine	71.6±2.1c	+4
L-serine	50.0±4.8d	+3
Basal medium (control)	31.6±2.7de	+1

Data represented above are means of three replicates ± SE at 5% level of probability. Values followed by different letters are significantly different by DMRT ($P \leq 0.05$).

greater biomass yield than ketohexose (fructose) under the same conditions (Table 2). This may be due to efficient incorporation of glucose directly into respiratory pathway after phospholylation. Similar observation on glucose utilization was reported by Jonathan (2002) for *Psathyrella atroumbonata* and *Schizophyllum commune*. The inability of oligosaccharides to produce high biomass may be linked to their molecular size and structure (Garraway and Evans, 1984).

Generally, complex sugars and sugar alcohol produce little biomass with the exception of dextrin and mannitol (Table 2). The little amount of mycelial production with polysaccharides and sugar alcohols may be attributed to their complex nature. Hydrolytic enzymes will be required to convert polysaccharides and sugar alcohols to simple sugars before they will enter respiratory pathways. The enhancement of moderate mycelial yield of *P. florida* by dextrin was similar to the observation made by (Jonathan and Fasidi 2001) on *P. atroumbonata*. Mannitol (a sugar alcohol) also supported good biomass yield of *P. florida* (Table 2). This result agrees with the suggestion of several authors (Guha and Banerjee, 1971; Chandra and Purkayastha, 1977; Fasidi and Akwakawa, 1996) that independently reported mannitol as the most suitable sugar alcohol for the growth of mushrooms. The preference of mannitol to other sugar alcohol may be due to the formation of fructose through oxidation and subsequent incorporation in respiratory pathway after phosphrylation (Mahier and Cordes, 1971).

All the twelve amino acids used in this study enhanced biomass production of *P. florida* (Table 3). The most stim-

Table 4. Influence of complex organic and inorganic nitrogen compounds on biomass production in *P. florida*.

Nitrogen Compounds	Biomass yield (mg/30 cm^3)	Mycelial density
Complex Organic Sources		
Casein hydrolysate	126.7±4.7b	+6
Peptone	90.0±3.3d	+5
Urea	38.3±2.9f	+1
Yeast Extract	153.3±5.7a	+7
Inorganic Sources		
Ammonium nitrate	100.0±3.9c	+5
Ammonium sulphate	63.3±1.3e	+3
Calcium nitrate	61.6±4.3e	+3
Potassium nitrate	90.0±2.3d	+5
Sodium nitrate	46.7±3.8f	+2
Basal medium (control)	35.0±3.8f	+1

Data represented above are means of three treatments ±SE at 5% level of probability. Values followed by the different letters are significantly different by DMRT ($P \leq 0.05$).

Table 5. Effect of different volumes of *Pleurotus florida* inoculum on biomass yield.

Volume of inoculum (cm^3)	Biomass yield (mg/100 cm^3)	Mycelial density
0.5	65.0±0.9h	+1
1.0	80.0±1.8h	+2
2.0	110.0±2.9g	+3
3.0	156.7±3.8f	+4
4.0	205.0±2.3	+5
5.0	246.0±4.8d	+5
6.0	285.0±3.9c	+6
7.0	330.0±3.3a	+8
8.0	303.3±3.7b	+7
9.0	276.7±5.3c	+6
10.0	230.0±4.3d	+4

Values are means of three replicates ± SE at 5% level of probability. Means followed by different letters are significantly different by DMRT ($P \leq 0.05$).

ulatory amino acid was tryptophan followed in order by alanine and glutamine ($P \leq 0.05$). The preference of tryptophan to other amino acids may be due to the ease at which it is being transported across the fungal cell remembrance. This result is similar to that obtained by Voltz (1972) on *Cantharellus cibarius* and *Mycothecium verucana*. Jonathan (2002) also observed alanine to be supportive for mycelial production in *Lepiota procera* as observed in this study.

In the series of complex and inorganic nitrogen sources (Table 4), it was observed that inorganic compounds supported moderate biomass production. The best biomass yield (100.0 mg/3 cm^3) was found with ammonium nitrate closely followed by potassium nitrate. This result is contrary to that obtained by (Jonathan and Fasidi, 2001) for *P. atroumbonata* where ammonium nitrate supported insignificant mycelial yield. Among the complex nitrogen compounds, yeast extract and casein hydrolysate supported significant biomass yield of 153.3 and 126.7 mg/30 cm^3 respectively. Similar utilization of yeast extract by higher fungi has been reported by Alberghina (1973) on *Neurospora crassa* by Fasidi and Olorunmaiye (1994) on *P. tuber-regium*. The stimulatory action of yeast extract on biomass yield of *P. florida* may be linked with its high carbohydrate, amino acids and vitamins composition (Nolan, 1970). The effect of different amounts of mycelial inoculum on biomass production is shown on Table 5. The biomass yield varied with the volume of inoculum but the differences were only significant ($P \leq 0.05$) when the volume was greater than 2.0 ml. The best biomass yield was obtained with 7.0 cm^3 of inoculum. The result obtainned is different from that reported by Jin-Zhong (2003 for *P. tuber-regium*.

It was clearly shown from this investigation that biom-

ass production in *P. florida* is supported by glucose, fructose, yeast extract, mannitol and a high concentration of inoculum. Therefore, we conclude that appropriate harvest time, inorganic and organic compounds and mass transfer are critical factors in optimizing biomass yield of *P. florida*. Further investigation on large scale fermentation and production of exopolysacharide are underway in order to optimize the yield of *P. florida*

REFERENCES

Alberghina FAM (1973). Growth relation in *Neurospora crassa*. Effect of Nutrients and Temperature. Arch. Microbiol. 89:83–94.

Alexopolous CJ, Mims CW, Blackwell M (1996). Introductory Mycology (4th Ed.) New York. John Wiley.

Alofe FR, Odu EA, Illoh HC (1998). Mushrooms, Man and Nature. Edible wiled mushrooms on decaying wood in Obafemi Awolowo University Campus. The Nigerian Fields. 63:3–18.

Chandra A, Purkayastha RP (1977). Physiological Studies on Indian Mushrooms. Trans. Br. Mycol. Soc. 69:63–70.

Fasidi IO (1996). Studies on *Volvariella esculenta* (Mass) Singer, Cultivation on Agricultural wastes and proximate composition of stored mushroom. *Food Chemistry*. 55(2): 161-163.

Fasidi IO, Akwakwa DO (1996). Growth requirements of *Volvariellas speciosa* (Fr.Ex.Fr.) Singer, as Nigerian Mushroom. Food Chem. 55:(2)165–168.

Fasidi IO, Olorunmaiye KS (1994). Studies on requirements for growth of *Pleurotus tuber-regium* (Fr.) Singer, a Nigerian edible mushroom. Food Chem. 50:397–401.

Garraway OM, Evans CR (1984). Fungi Nutrition and Physiology. John Wiley and Sons. New York. Toronto Singapore.

Gbolagade JS, Fasidi IO, Ajayi EJ, Sobowale AA (2006). Effect of Physico-chemical factors and semi synthetic media on vegetative growth of *Leutinus submudus* (Berk.) an edible mushroom from Nigeria. Food Chem. pp. 742-747.

Griffin DH (1994). Fungal Physiol. (2nd Ed.) New York: Wiley Liss.

Guha AK, Banerjee (1971). Effect of different carbon compounds on submerged production of *Agaricus campestris*. J. Food Sci. Tech. 8:82–83.

Hudson HJ (1992). Fungal Biology (1st Ed). Cambridge University Press. Great Britain.

Jin–Zhong W, Cheung PCK, Ksa-Hing W, Naian-Lai H (2003). Studies on submerged fermentation of *Pleurotus tuber-regium* (Fr.) Singer part D physical and chemical factors affecting the rate of mycelial growth and bioconoersion efficiency. Food Chem. 81:389–393.

Jonathan SG (2002). Vegetative Growth Requirements and Antimicrobial activities of some higher fungi in Nigeria. Ph.D Thesis, University of Ibadan, Ibadan. Nigeria.

Jonathan SG, Fasidi IO (2001). Effect of carbon, Nitrogen and Mineral Sources on growth of *Psathyenella atroumbonata* (Pegler), a Nigerian Edible Mushroom. Food Chem. 72:479-483.

Jonathan SG, Fasidi IO (2003). Studies on *Psathyerella atroumbonatai* (Pegler), a Nigerian edible fungus. Food Chem. 81:481–484.

Jonathan SG; Fasidi IO, Ajayi EJ (2004). Physic-chemical Studies on *Volvariella esculenta* (Mass Singer, a Nigerian Edible Fungus. Food Chem. 85:339–342.

Kadiri M, Fasidi IO (1994). Growth requirements of *Lentinus subnudus* (Berk.), a Nigerian edible mushroom. *Chemie Microbiol*. Technol. Lebensm. 16(314):80–84.

Mahier HR, Cordes EH (1971). Biological Chemistry (2nd Ed.) New York Hamper and Row.

Maziero R, Cavazzoni V, Lucia V, Bononi R (1999). Screening of Basidiomycetes for production of Exopolysaccharides and Biomass in Submerged culture. Revista. Microbiogia. 30: 77–84.

Nolan RA (1970). The phycomycete *Catenaria anguillulue* growth requirements. J. Gen. Microbiol. 60:167–180.

Snedecor GW, Cochran GW (1987). Statistical Methods .Oxford IBH Publishing Co. Ltd, New Delhi, pp. 20-25.

Voltz PA (1972). Nutritional studies on species and mutants *Leposta, Cantharellus* and *Volvariella*. Mycopath. Mycol. Appl. 48:175–185.

Zoberi MH (1972). Tropical Macrofungi. Macmillan Press. London.