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Full Length Research Paper

The effect of different nutrient sources on biomass production of *Lepiota procera* in submerged liquid cultures

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The effect of various organic, inorganic and complex compounds on the biomass production (mycelial dry weight) of *Lepiota procera*, a Nigerian edible higher fungus was investigated. Among the seventeen carbon compounds tested, mannose enhanced the best biomass yield. This was followed in order by glucose, raffinose and fructose (P>0.01) while Myo-inositol, sucrose and dextrin were the least stimulatory. Of all the twenty-one nitrogen sources used, D-alanine was the most utilizable. Moderate biomass yield was also supported by yeast extract and peptone while the least biomass production was recorded with D-cysteine and sodium nitrate. The carbon/nitrogen ratio that sustained the best biomass yield was 4:1 while the least utilized ratios were 2:5 and 3:5 respectively. Magnesium and calcium were the best macronutrients. Likewise, trace elements (Mn and Zn) stimulated very good mycelial growth.

Key words: Biomass, *Lepiota procera*, nutrients, submerged culture.

INTRODUCTION

Lepiota procera (Fr.) S.F. Gray, commonly called the parasol mushroom, is widely distributed in nature. This mushroom, which belongs to phylum basidiomycota, order agaricales and family Lepiotaceae has been reported in Africa, America, Europe and South-East Asia (Pegler, 1983; Singer, 1986; Alexopoulous et al., 1996). *L. procera,* which is known for its succulent qualities, has a very large sporocarp and stately appearance. The pileus is about 10–20 cm in diameter, at first globuse, expanding to convexo campanulate. It is covered with shaggy brown scales with a white background (Jonathan, 2002). The gills are free, remote from the stem, white, but, pinkish in old matured fruit bodies. The stipe is cylindrical, and long (10–20 cm diameter), annulus is persistent and movable while the spore prints are creamy white (Singer, 1986; Jonathan, 2002).

Lepiota species are quite edible but unfortunately, they superficially resemble certain species of *Amanita,* which are extremely poisonous. They possess white spores, free gills and annuli, but unlike Amanita, *L. procera* lacks volva and the annulus is usually persistent and conspicuous. In Nigeria, people always avoid *L. procera* growing in the wild because of fear of being poisoned by closely related poisonous species. Jonathan and Fasidi (2003) carried out investigations on nutrient requirements of *Tricholoma lobayensis* grown in a submerged culture; and they reported that fructose, asparagine, potassium and Zinc were required for the optimal biomass production of this fungus Therefore, this present study was aimed at investigating conditions necessary for biomass production in *L. procera*. This will provide useful information about cultivation biotechnology of *L. procera* in Nigeria.

MATERIALS AND METHODS

L. procera mycelia were obtained by tissue culture method as described by Jonathan and Fasidi (2001) and maintained on PDA enriched with 0.5% yeast extract. The biomass production in this fungus was determined by a mycelial dry weight method described by Fasidi and Olorunmaiye (1994). The ingredients required to form

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Table 1. Effect of different carbon sources on biomass production in *Lepiota procera*

Carbon source	Biomass yield (mg/30 cm^3)	Final pH
Control (basal medium)	40.0g	7.6
Monosaccharides		
Arabinose	83.3c	5.7
Fructose	123.3cd	6.2
Galactose	70.0ef	4.9
Glucose	170.0ab	6.3
Mannose	183.3a	6.7
Sorbose	51.7g	7.3
Rhamnose	73.3ef	7.1
Oligosaccharides		
Cellobiose	75.0ef	6.5
Lactose	53.3g	5.7
Maltose	60.0fg	6.8
Raffinose	136.7c	6.1
Sucrose	43.3gh	5.5
Sugar alcohol		
Mannitol	53.3g	5.6
Myo-iInositol	35.0h	5.8
Polysaccharides		
Cellulose	75.0ef	6.7
Dextrin	46.7gh	5.6
Soluble starch	43.3gh	6.3

Means followed by the same letter(s) are not significantly different (P>0.01) by Duncan's Multiple Range Test.

the basal medium and streptomycin sulphate were dissolved in 1000 cm^3 of deionised water and adjusted to a pH of 6.5. The medium was dispensed into 150 cm^3 bottles (30 cm^3 per bottle), which were sealed with aluminum foil and autoclaved at 121°C for 15 min. After cooling, each bottle was inoculated with a 7 mm diameter mycelial disc of 5 day old fungus and incubated at a room temperature of 30±2°C for 7 days. Biomass production was determined using the procedure of Chandra and Purkayastha (1977).

Carbon nutrition

The basal medium used contained peptone (2.0 g), KH_2PO_4 (0.5 g), $MgSO_4.7H_2O$ (0.5 g), and 1000 cm^3 of deionised water. The medium was supplemented separately with 1% carbon of each carbon source. For starch, dextrin and cellulose, approximately 10 g/1000 cm^3 was used while the control medium was constituted without any carbon compounds.

Nitrogen nutrition

The basal medium consisted of KH_2PO_4 (0.5 g), $MgSO_4.7H_2O$ (0.5 g), thiamine hydrochloride (500 µg), fructose (10 g) and 1000 cm^3 of deionised water. The amount of nitrogen in each supplemented compound was 0.1% nitrogen while peptone, urea, yeast extract, malt extract and casein were supplemented at the rate of 2 g/1000cm^3.

Carbon and nitrogen ratio

A mixture of 0.15 g/1000 cm^3 of mannose and alanine (served as C:N ratio of 1:1, while other ratios were prepared proportionately. The basal medium was similar to that used for investigating nitrogen nutrition except that fructose was omitted.

Macro elements

The basal medium used was that described by Chandra and Purkayastha (1977). To investigate effects of macronutrients on growth, Na, K, Mg and Ca compounds in the liquid medium were replaced by their ammonium radicals (e.g. $NaNO_3$ was replaced by NH_4NO_3 while $MgSO_4$ was replaced by $(NH_4)_2SO_4$). Two sets of controls were employed. Control 1 had all the macro elements while control 2 had none.

Trace elements

The trace elements studied were Cu, Fe, Mn, Zn and Co (in their sulphate forms). The basal medium was the same as for macro elements while the trace elements tested were omitted from the medium. Complete medium (basal medium plus all the trace elements) and basal medium were used as controls.

Analysis of data

The data obtained from these studies were analyzed using Analysis of Variance (ANOVA) while treatment means were separated using Duncan's Multiple Range Test (DMRT) (Snedecor and Cochran, 1987).

RESULTS AND DISCUSSION

All the tested carbohydrate sources except myoinositol significantly enhanced biomass yield in *L. procera* (P>0.01) while mannose was the most utilizable carbon compound followed by glucose and raffinose respectively (Table 1). Similar utilization of mannose and glucose were reported by Chandra and Purkayastha (1977) (on *Agaricus campestris* and *Volvariella volvaceae*) and Jonathan and Fasidi (2001) (on *Psathyrella atroumbonata*). Mannose and glucose were reported as good substrates in cellular respiration (Sistrom and Machilis, 1955; Griffin, 1994). The enhancement of biomass production by raffinose (an oligosaccharide) suggests that *L. procera* possesses hydrolytic enzymes, which catalyse the hydrolysis of raffinose to an assimilatable monosaccharide, which could support its growth (Carlie and Watkinson, 1996). The poor growth of this fungus may be linked to toxic effects of myoinositol on fungal cell walls (Garraway and Evans, 1984).

D-alanine sustained the best growth among the tested nitrogen compounds (P>0.01) (Table 2). This result correlates with the reports of Chandra and Purkayastha (1977) (on *Volvariella volvaceae*). The preference of alanine over other nitrogen compounds may be due to the ease at which it is being transported across the fungal

Table 2. Effect of different Carbon sources on biomass production in *Lepiota procera.*

Nitrogen source	Biomass yield (mg/30 cm^3)	Final pH
Control	40.0g	6.8
Inorganic Nitrogen Compounds		
$NaNO_3$	35.0gh	6.2
KNO_3	53.3fg	5.8
$Ca(NO_{3)2}$	50.0fg	5.4
NH_4NO_3	48.3fg	6.5
$(NH_4)_2SO_4$	78.3c	7.0
Amino Acids		
L – aspartic acid	56.7f	7.7
L – asparagine	53.3f	5.9
D – alanine	150.0a	6.8
L – glutamic acid	133.3b	6.4
L – glutamine	60.0cf	6.3
D – cysteine	33.3gh	6.5
DL – methionine	58.3f	6.6
L – tryptophan	80.0e	6.1
DL – phenyl alanine	100.0d	6.7
DL – leucine	41.7g	6.2
L – lysine	73.3e	5.7
Complex Nitrogen Compounds		
Casein	56.7f	7.1
Malt extract	83.3e	6.6
Peptone	123.3c	7.2
Urea	46.7fg	7.5
Yeast extract	120.0c	6.7

Means followed by the same letter(s) are not significantly different (P>0.01) by Duncan's Multiple Range Test.

Table 3. Effect of different carbon/nitrogen ratio (C:N) on production of *Lepiota procera.*

Carbon:Nitrogen ratio	Biomass yield (mg/30 cm^3)	Final pH
Control (basal medium)	**43.3 gh**	**6.5**
1:1	60.0 cf	6.9
1:2	43.3 gh	6.3
1:3	40.0 gh	5.9
1:4	41.7 gh	7.1
1:5	33.3 h	5.3
2:1	70.0 c	5.7
2:3	53.3 h	5.8
2:5	33.3 h	5.9
3:1	116.7 bc	6.1
3:2	36.7 h	6.4
3:4	51.7 h	6.3
3:5	36.7 h	5.7
4:1	160.0 a	6.5
4:3	100.0 c	6.3
4:5	56.7 f	6.2
5:1	126.7 b	6.4
5:2	93.3 cd	6.6
5:3	75.0 c	5.8
5:4	68.3 c	6.7

Means followed by the same letter(s) are not significantly different (P>0.01) by Duncan's Multiple Range Test.

cell membrane (Griffin, 1994). L-glutamic acid and peptone supported biomass yields of 133.3 and 123.3 $mgcm^{-3}$ respectively. This result is similar to that obtained by Voltz (1972) (on *Cantharellus clavatus* and *Myrothecium verucaria*). The utilization of peptone may be due to its carbon and amino acid composition while that of glutamic acid may be linked to its role in the glycolytic pathway (Garraway and Evans, 1984).

Carbon/nitrogen ratios significantly affected biomass yield of *L. procera* and optimal growth was induced by the ratio of 4:1, followed by 5:1 and 3:1 respectively (Table 3). The result suggests that *L. procera* utilizes agricultural substrates that contain carbohydrate and nitrogen sources within a tolerable concentration. The best C:N ratio for *L. procera* is different from that obtained by Chandra and Purkayastha (1977) (for *A. campestris*) and Fasidi and Jonathan (1994) (for *V. esculenta*). This indicates that C:N ratios required by each mushroom species may differ. The basal medium supplemented with Ca, Mg, K and Ma (Complete medium) significantly enhanced greater biomass production of *L. procera* (Table 4a) suggesting that all macro elements tested are essential for the growth of this fungus. This result is similar to those reported by Humfeld and Sugihara (1952) and Fasidi and Olorunmaiye (1994). The magnesium free medium supported the least growth whereas the sodium free medium enhanced very good growth. This implied that Mg was the most utilized macro element while Na was the least. Similar utilization of Mg, and Na were reported by Fasidi and Jonathan (1994), Fasidi and Olorunmaiye (1994) and Voltz (1972) on *V. esculenta, P. tuber-regium* and *C. cibarius*, respectively. Magnesium is important because it is very useful in enzyme activation and ATP metabolism (Griffin, 1994).

For microelements, the basal medium containing Co, Cu, Fe, Mn and Zn (complete medium) inhibited the growth of *L. procera* (Table 4b). This shows that all the tested trace elements are not required for biomass production in this fungus. Cobalt free medium significantly enhanced growth (P>0.01), showing that Fe, Mn, Zn and Cu are stimulatory while Co is inhibitory. However, Mn free medium induced the least biomass yield among the tested micronutrients (Table 4b). This stimulatory effect of Mn may be linked to its role in TCA cycle and nucleic acid synthesis (Garraway and Evans, 1984).

Table 4. Effect of different mineral elements on biomass production of *Lepiota procera*.

Mineral elements		Biomass yield (mg/30 cm^3)	Final pH
(a)	**Macro elements**		
	Basal medium (control 1)	40.0d	6.5
	Complete medium minus Ca	66.7bc	6.2
	Complete medium minus K	78.3b	6.3
	Complete medium minus Mg	46.7d	5.8
	Complete medium minus Na	103.3a	6.4
	Complete medium (control 2)	110.0a	5.9
(b)	**Micronutrients**		
	Basal medium (control 1)	40.0c	6.4
	Complete medium minus Co	103.3a	6.2
	Complete medium minus Cu	83.3b	6.3
	Complete medium minus Fe	85.3b	6.6
	Complete medium minus Mn	55.0d	5.8
	Complete medium minus Zn	76.7c	5.9
	Complete medium (control 2)	66.7cd	6.7

Means followed by the same letter(s) are not significantly different (P>0.01) by Duncan's Multiple Range Test.

In conclusion, this study, showed that various carbon, nitrogen and C:N ratios significantly enhanced biomass yield in *L. procera*. Therefore, the medium used to propagate high yield starter culture mycelia of *L. procera* should be enriched with mannose and D-alanine in a ratio of 4:1. It is also important to analyze agricultural wastes used for the growing of this fungus to ascertain that they are not deficient in Mg, Ca, Mn and Zn, which are essential for the growth of *L. procera*.

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