

## Studies on Biomass Production in *Auricularia polytricha* Collected from Wilberforce Island, Bayelsa State, Nigeria

<sup>1</sup>S.G. Jonathan, <sup>2</sup>D.D.S. Bawo, <sup>1</sup>D.O. Adejoye and <sup>2</sup>O.F. Briyai

<sup>1</sup>Department of Botany and Microbiology, University of Ibadan, Ibadan Nigeria

<sup>2</sup>Department of Biological Sciences, Niger Delta University,  
Wilberforce Island, Bayelsa State, Nigeria

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**Abstract:** *Auricularia polytricha* (Mont) Saccardo, were found growing on decaying logs of wood in their large numbers within the marshy riverine ecosystem area of Wilberforce Island, Bayelsa State, Nigeria. The sporophores of this edible mushroom were tissue cultured and biomass production in submerged liquid medium was assessed. This fungus produced best mycelial biomass ( $340 \text{ mg cm}^{-3}$ ) at  $25^\circ\text{C}$  after 15 days of incubation while no biomass was produced at 5, 45 and  $50^\circ\text{C}$  after 20 days. Likewise, very good biomass was produced at pH 6.5 after 10 days of incubation. Among the carbohydrate sources tested, glucose stimulated highest biomass yield of  $375 \text{ mg cm}^{-3}$  at 1.6% concentration closely followed by fructose ( $350 \text{ mg cm}^{-3}$ ) at 1.8% concentration. All the nitrogen compounds investigated enhanced significant biomass production ( $p \leq 0.05$ ). However, 0.8% of peptone stimulated the best biomass yield ( $320 \text{ mg cm}^{-3}$ ) followed by tryptophan ( $300 \text{ mg cm}^{-3}$ ) at 0.9% concentration. The volume of inoculum of *A. polytricha* that produced highest biomass of this fungus in liquid culture was  $7.0 \text{ cm}^3$ . The implications of these findings were discussed.

Keywords: Biomass, *Auricularia polytricha*, Wilberforce Island, collection, submerged culture

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### INTRODUCTION

Previous work have been carried out on collection, identification and isolation of indigenous Nigerian mushrooms<sup>[1, 2, 3, 4, 5]</sup>. Investigations were carried out on these fungi basically, to determine their nutritional requirements and utilization in biotechnological processes. These organisms have been implicated in lignin and other recalcitrant substances degradation, soil bioremediation, production of edible biomass and secondary metabolites<sup>[6, 7, 8, 9]</sup>. *Auricularia polytricha* is widely distributed throughout the tropical and sub-tropical regions of the world<sup>[10, 27]</sup>. It belongs to phylum basidiomycota, order auriculariales and family auriculariaceae<sup>[11]</sup>. This edible mushroom species is the commonest among the jelly-like fungi in West Africa. It grows wildly during the rainy season within the bark of a decaying wood under shade<sup>[3, 10, 11]</sup>. The sporophores of this fungus are usually found in their large numbers during late July.

*Auricularia polytricha* has a very peculiar consistency. The basidiocarp when fresh is rubbery, gelatinous and ear-like in structure but when dried, it is shapeless and brittle. Its edible fruitbodies could be easily identified by pilose upper surface which is

strongly capitate with dark brown smooth hymenium<sup>[3, 10]</sup>. Fungal biomass have been found to be important for several purposes (i) It could be of immense advantage for process reduction in fermentation technology<sup>[12]</sup> (ii) Fungal biomass could be used as food or protein supplement<sup>[13]</sup> (iii) Fungal biomass could be used for flavour extraction<sup>[14]</sup>. (iv) They could be used for extraction of metabolites such as polysaccharides and enzymes<sup>[15]</sup> (v) They could also be used for wound treatment<sup>[16]</sup>.

The primary objective of the present studies is therefore to culture *A. polytricha* in sub-merged liquid medium under different physico-chemical parameters with the aim of producing high yield biomass of this fungus.

### MATERIALS AND METHODS

**Micro-organism:** *Auricularia polytricha* (Mont) Saccardo were found growing on logs of decaying wood in their large numbers within the marshy riverine ecosystem in Amassoma, Wilberforce Island, Bayelsa State, Nigeria. The sporophores of this fungus were tissue cultured and the mycelia culture thus obtained were maintained on Malt extract agar (Difco)<sup>[2, 17]</sup>.

**Effect of incubation temperature on biomass production:** The effect of incubation temperature on biomass production in *A. polytricha* was determined in a chemically defined medium. This medium has the following compositions (g L<sup>-1</sup>) glucose 10.0, yeast extract 3.0, K<sub>2</sub>HPO<sub>4</sub> 0.6, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.3, 1L of distilled water and pH of 6.20<sup>[17]</sup>. The basal medium was dispensed into 250cm<sup>3</sup> conical flasks (100cm<sup>3</sup> per flask). Each was covered with aluminum foil and sterilized in the autoclave at 1.02 kg cm<sup>-2</sup> at 121°C for 15 min. After cooling, each flask was inoculated with vigorously growing (5 day old fungus) mycelial disc (7.0 mm diameter) acid incubated at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C respectively for 5, 10, 15 and 20 days. Each treatment was replicated three times. The mycelial biomass produced were harvested using the method of Gbolagade *et al*<sup>[4]</sup>.

**Effect of pH on biomass production in *A. polytricha*:** For pH, the same basal medium used for temperature determination was employed. The medium pH was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.5. 100 cm<sup>3</sup> of each treatment was dispensed into 250 cm<sup>3</sup> conical flasks and replicated thrice. They were autoclaved at 121°C and 1.02 kg cm<sup>-2</sup> for 15 min. After cooling, they were inoculated and biomass produced were harvested as described in the temperature experiment.

**Utilization of different concentration of carbon compounds for biomass production in *A. polytricha*:** Four carbon sources namely glucose, fructose, mannitol and cellulose were evaluated. The concentration of these carbohydrate compounds used varies between 0.2-2.0% while the basal medium that lack any carbon compound (0%) served as the control. The fermentation medium has the following compositions in percentage (%): peptone 0.2, MgSO<sub>4</sub>. 7H<sub>2</sub>O 0.1, K<sub>2</sub>H PO<sub>4</sub>. 3H<sub>2</sub>O 0.1 and 1000 cm<sup>3</sup> of distilled water<sup>[18]</sup>. Each concentration of these carbon compounds was supplemented in the basal medium and the experiment were replicated three times.

**Utilization of different concentration of nitrogen compounds for biomass production in *A. polytricha*:** Four different sources of organic and inorganic nitrogen sources were used. These were alanine, tryptophan, ammonium sulphate and peptone. Fermentation medium was similar to the one of carbon sources but the nitrogen sources were replaced at an equivalent concentration. Various concentration of nitrogen compounds supplemented in this medium ranges from 0.01-0.12 (Table 4).

**Effect of inoculum sizes on biomass production in *A. polytricha*:** The basal medium used was that described by Maziero *et al.* (1999). It has the following composition (g L<sup>-1</sup>) peptone 1.0 yeast extract 2.0, K<sub>2</sub>HPO<sub>4</sub> 1.0, MgSO<sub>4</sub>. 7 H<sub>2</sub>O 0.2, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 5.0, glucose 39.0 and 1000 cm<sup>3</sup> of distilled water, the medium was adjusted to pH 6.5. To generate enough inoculum, *A. polytricha* was initially inoculated onto the above medium for 6 days<sup>[2]</sup>. Thereafter, different concentrations of this fungus (0.5-10.0 cm<sup>3</sup>) (Table 5), were then aseptically inoculated into 250 cm<sup>3</sup> conical flasks containing 100 cm<sup>3</sup> of the chemically defined liquid medium. Each treatment has 3 replicates. Incubation was carried out for 10 days at room temperature 30±2°C after which mycelia were harvested as described in the previous experiment.

**Analysis of data:** Rating results in each treatment of triplicate experiments were subjected to analysis of variance (ANOVA) using general linear model option SAS. Test of significance were determined by Duncan's multiple range test at 0.5% level of probability.

## RESULTS AND DISCUSSION

Table 1 shows that *A. polytricha* could produced mycelial biomass within the temperature range of 10 and 40°C. The best biomass yield was obtained at 25°C after 15 days of incubation. It could be seen clearly that the minimum, optimum and maximum temperatures for biomass production of this fungus were 10, 25 and 40°C respectively. It was also observed that no biomass was produced at 5, 45 and 50°C. Biomass production of this fungus was not favoured by extremely cold nor high temperatures (5,45and 50°C). Similar observations were made by Gbolagade *et al.*<sup>[4]</sup> for

Table 1: Effect of Incubation temperature on biomass production in *A. polytricha*

Day Temp (°C)	Biomass production (mg 100 <sup>-1</sup> ) cm <sup>3</sup>			
	5	10	15	20
5	-	-	-	-
10	-	30e	40e	40ef
15	20d	70d	40e	30f
20	40cd	120c	70d	55de
25	90a	295a	340a	270a
30	65b	260b	300b	240b
35	30de	120c	125c	90c
40	10e	20f	20f	20g
45	-	-	-	-
50	-	-	-	-

Data represented above are means of 3 replicates. Values followed by the same letter(s) along each vertical column are not significantly different by Duncan's multiple range test (p≤0.05)

Table 2: Effect of pH on biomass production in *A. polytricha*

pH Day	Biomass production (mg 100 cm <sup>-3</sup> )			
	5	10	15	20
4.0	-	-	-	-
4.5	30g	30e	50h	40gh
5.0	65f	120d	100fg	50fg
5.5	90e	190c	150e	90de
6.0	145b	265b	200d	150c
6.5	190a	310a	240b	165bc
7.0	120c	260b	270a	190a
7.5	100dc	205c	220c	150c
8.0	70f	130d	90g	75e
8.5	20g	40e	50h	30h
9.0	-	-	-	-

Data represented above are means of 3 replicates. Values followed by the same letter along each vertical column are not significantly different by Duncan's multiple range test (p≤0.05)

*Pleurotus florida*. Maziero *et al.*<sup>[12]</sup> also observed that the best biomass yields were obtained between temperature range of 25 and 30°C.

It was observed from Table 2 that *A. polytricha* produced best biomass (310 mg/100 cm<sup>3</sup>) at pH 6.5 after 10 days of incubation. The least mycelial biomass (20.0 g/100 cm<sup>3</sup>) was obtained after 5 days of culturing while no biomass was produced at pH 4.0 and 9.0 respectively. Biomass yield on the 5th day was generally lower than that of the 10, 15 and 20 days. The reason for this may be due to the fact that on the 5th day, the fungal mycelia have not fully recovered from the logarithm phase. During this phase, there may be no obvious sign of metabolism and development. Microorganisms generally start to generate energy for anabolism, growth and biomass production<sup>[19]</sup>. At the 10th day, the fungus was experiencing exponential phase. This is the period of active metabolism, growth, development and optimal biomass production by this fungus<sup>[19]</sup>. On the 15th and 20th day, it was undergoing declining phase. This may be the reason why low biomass yields were generally observed after 15th and 20th day. These results were similar to that of Chandra and Purkayastha<sup>[20]</sup> on *Agaricus compestis*. Excellent mycelial biomass yield were also obtained by Jonathan and Fasidi<sup>[21]</sup> on *Psathyrella atroumbonata* at pH 6.5.

Table 3 demonstrates the effects of carbon compounds for mycelial biomass production. Glucose, a monosaccharide at the concentration of 1.6% produced the best biomass yield (375.0 mg/cm<sup>3</sup>). Also, it was observed that monosaccharides in general were better than sugar alcohol and complex sugar (cellulose) for biomass production. This was probably due to ease in polymerization and their simple nature<sup>[17]</sup>. These observations in regard to *A. polytricha* seemed to be important because only little is known on the utilization of carbon compounds for biomass production in

Table 3: Utilization of different concentrations of carbon compounds for biomass production in *A. polytricha*

Carbon compounds concentration (%)	Biomass production (mg 100 cm <sup>-3</sup> )			
	glucose 5	fructose 10	mannitol 15	cellulose 20
0%	25j	25k	25j	25k
0.2%	50i	35jk	45i	50ij
0.4%	75h	60i	80g	65gh
0.6%	110g	70hi	85fg	70fg
0.8%	170f	95g	110de	90de
1.0%	205e	120f	180bc	175a
1.2%	270d	155c	215a	140b
1.4%	340b	200d	170c	100c
1.6%	375a	290bc	100e	85ef
1.8%	305c	350a	75g	60hi
2.0%	210e	280c	55hi	40j

Data represented above are means of 3 replicates. Values followed by the same letter along each vertical column are not significantly different by Duncan's multiple range test (p≤0.05)

Table 4: Utilization of different concentrations of nitrogen compounds for biomass production in *A. polytricha*

N <sub>2</sub> Compounds Concentration in %	Biomass production (mg/100cm <sup>3</sup> )			
	Alanine	Tryptophan	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Peptone
0	20h	20k	20h	20k
0.01	70f	40j	50ef	30jk
0.02	100e	60ij	50ef	40ij
0.03	150d	75hi	120ab	70h
0.04	190bc	95g	110b	100g
0.05	230a	120f	90cd	160f
0.06	180c	170e	75d	210d
0.07	155d	240d	70d	275c
0.08	115e	290bc	45f	320a
0.09	70f	285ab	40fg	290bc
0.10	50g	300a	30gh	210d
0.12	50g	250de	30gh	185ef

Values followed by the same letter(s) along each column are not significantly different by Duncan's multiple range. Data are means of 3 replicates

*A. polytricha*. The little amount of biomass with cellulose may be attributed to its complex nature. Sugar alcohol and polysaccharides will be hydrolyzed to monosaccharide before they will enter respiratory pathways<sup>[22]</sup>.

All the 4 nitrogen compounds used in this investigation significantly promoted biomass yield. (Table 4). The most stimulatory nitrogen compound was peptone at 0.08% concentration. This result is contrary to that obtained by Gbolagade *et al.*<sup>[4]</sup> on *Pleurotus florida*. These workers obtained better biomass yield in ammonium nitrate better than peptone. The differences in nitrogen sources requirements may suggest that biomass production in different fungi may be influenced by different nutritional requirements. It was also observed that very low concentration of nitrogen compounds (0.01-0.03%) generally supported little biomass yield while low concentration 0.4% and above were supportive to high biomass yield<sup>[23]</sup>. The

Table 5: Effect of inoculum sizes on biomass production in *A. polytricha*

Volume of inoculum (cm <sup>3</sup> )	Biomass yield mg 100 cm <sup>-3</sup>	Mycelial density
0.5	25j	+2
1.0	40ij	+3
2.0	65h	+4
3.0	85g	+4
4.0	110f	+7
5.0	120ef	+7
6.0	175c	+8
7.0	260a	+9
8.0	200b	+7
9.0	180c	+5
10.0	150d	+4

Values followed by the same letters(s) along each column are not significantly different by Duncan's multiple range test ( $p \leq 0.05$ ). Data are means of 3 replicates

best yield (320 mg/100cm<sup>3</sup>) was obtained with peptone. Similar utilization of peptone by basidiomycetes has been reported<sup>[24]</sup>. The supportive action of peptone on biomass production in *A. polytricha* may be linked to its carbohydrate, amino acids and vitamin composition.

The effect of inoculum sizes on biomass production is presented on Table 5. It was observed that all the volume of inoculum used (0.5-10.0) produced varying degrees of biomass yield. The best mycelial biomass was obtained at 7.0 cm<sup>3</sup> (i.e., 260 mg 100cm<sup>-3</sup>). This result is similar to that obtained by Jonathan *et al.*<sup>[25]</sup> for *L. subnudus*. Rew *et al.*<sup>[26]</sup> reported that the inoculum sizes may determine biomass production in an agitated system.

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