Studies on Biomass Production in *Auricularia polytricha* Collected from 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 mg cm$^{-3}$) at 25°C after 15 days of incubation while no biomass was produced at 5, 45 and 50°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 mg cm$^{-3}$ at 1.6% concentration closely followed by fructose (350 mg cm$^{-3}$) at 1.8% concentration. All the nitrogen compounds investigated enhanced significant biomass production ($p<0.05$). However, 0.8% of peptone stimulated the best biomass yield (320 mg cm$^{-3}$) followed by tryptophan (300 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 cm$^3$. The implications of these findings were discussed.

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

INTRODUCTION

Previous work have been carried out on collection, identification and isolation of indigenous Nigerian mushrooms [1, 2, 3, 4, 5]. 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 [6, 7, 8, 9]. *Auricularia polytricha* is widely distributed throughout the tropical and sub-tropical regions of the world [10, 27]. It belongs to phylum basidiomycota, order auriculariales and family auriculariaceae [11]. 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 [3, 10, 11]. 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 capitately with dark brown smooth hymenium [3, 10]. Fungal biomass have been found to be important for several purposes (i) It could be of immense advantage for process reduction in fermentation technology [12] (ii) Fungal biomass could be used as food or protein supplement [13] (iii) Fungal biomass could be used for flavour extraction [14] (iv) They could be used for extraction of metabolites such as polysaccharides and enzymes [15] (v) They could also be used for wound treatment [16].

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) [2, 17].
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⁻¹): glucose 10.0, yeast extract 3.0, K₂HPO₄ 0.6, MgSO₄.7H₂O 0.3, 1L of distilled water and pH of 6.20. The basal medium was dispensed into 250cm³ conical flasks (100cm³ per flask). Each was covered with aluminum foil and sterilized in the autoclave 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.⁴¹. Incubation was carried out for 10 days at room temperature 30±2°C after which mycelia were harvested as described in the previous experiment.

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 cm³ of each treatment was dispensed into 250 cm³ conical flasks and replicated thrice. They were autoclaved at 121°C and 1.02 kg cm⁻² 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, MgSO₄ 7H₂O 0.1, K₂HPO₄ 3H₂O 0.1 and 1000 cm³ of distilled water.⁴² 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.⁴⁷. It has the following composition (g L⁻¹): peptone 1.0 yeast extract 2.0, K₂HPO₄ 1.0, MgSO₄ 7 H₂O 0.2, (NH₄)₂SO₄ 5.0, glucose 39.0 and 1000 cm³ 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. Thereafter, different concentrations of this fungus (0.5-10.0 cm³) (Table 5), were then aseptically inoculated into 250 cm³ conical flasks containing 100 cm³ 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.⁴⁴ for A. polytricha.

| Day | Temp (°C) | 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  |           | 63b| 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).

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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, MgSO₄ 7H₂O 0.1, K₂HPO₄ 3H₂O 0.1 and 1000 cm³ of distilled water. Each concentration of these carbon compounds was supplemented in the basal medium and the experiment were replicated three times.

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| Day | Temp (°C) | 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  |           | 63b| 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  | 5   | 10  | 15  | 20  |
|-----|------|-----|-----|-----|-----|
| 4.0 | -    | -   | -   | -   | -   |
| 4.5 | 30g  | -   | 30e | 50h | 40h |
| 5.0 | 65f  | 120d| 100g| 50f | -   |
| 5.5 | 90e  | 190e| 150e| 90d | -   |
| 6.0 | 145b | 265b| 200d| 150c| -   |
| 6.5 | 190a | 310a| 240b| 165bc| -   |
| 7.0 | 120c | 260b| 270a| 190a| -   |
| 7.5 | 1000c| 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.*[12] 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 cm³) at pH 6.5 after 10 days of incubation. The least mycelial biomass (20.0 g/100 cm³) 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[19]. 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[19]. 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[20] on *Agaricus compexis*. Excellent mycelial biomass yield were also obtained by Jonathan and Fasidi[21] on *Psathyrella atroumbonata* at pH 6.5.

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

| Carbon compounds concentration (%) | Biomass production (mg 100 cm⁻³) |
|-----------------------------------|----------------------------------|
| glucose 5 | fructose 10 | mannitol 15 | cellulose 20 |
| 0%       | 25j         | 25k           | 25j         | 25k         |
| 0.2%     | 50i         | 35jk           | 45i         | 50j         |
| 0.4%     | 75h         | 60i           | 80g         | 65h         |
| 0.6%     | 110g        | 70hi          | 85fg        | 70g         |
| 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         | 100g        | 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*

| N₄ Compounds | Concentration in % | Biomass production (mg/100cm³) |
|--------------|--------------------|--------------------------------|
| Alanine      | 0.01               | 20h                           |
|              | 0.02               | 100e                          |
|              | 0.03               | 150d                          |
|              | 0.04               | 190bc                         |
|              | 0.05               | 230a                          |
|              | 0.06               | 180c                          |
|              | 0.07               | 155d                          |
|              | 0.08               | 115e                          |
|              | 0.09               | 70f                           |
|              | 0.10               | 50g                           |
|              | 0.12               | 50g                           |
| Tryptophan   | 20k                |                               |
|              | 20k                |                               |
|              | 20k                |                               |
|              | 20k                |                               |
| (NH₄)₂SO₄     | 50ef               |                               |
| Peptone      | 20k                |                               |
|              | 20k                |                               |
|              | 20k                |                               |

Values followed by the same letter(s) along each column are not significantly different by Duncan’s multiple range test. 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[22].

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.*[4] 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[23]. The
Table 5: Effect of inoculum sizes on biomass production in A. polytricha

| Volume of inoculum (cm$^3$) | Biomass yield mg in 100 cm$^3$ | 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<0.05). Data are means of 3 replicates.

best yield (320 mg/100cm$^3$) was obtained with peptone. Similar utilization of peptone by basidiomycetes has been reported\(^{24}\). 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$^3$ (i.e., 260 mg 100cm$^{-3}$). This result is similar to that obtained by Jonathan \(et \ al\(^{25}\) for L. subnudus. Rew \(et \ al\(^{26}\) reported that the inoculum sizes may determine biomass production in an agitated system.

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