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Effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata* (Pegler), a Nigerian edible mushroom

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Abstract

Effect of simple organic, inorganic and complex compounds on growth of *Psathyerella atroumbonata* (Pegler), a Nigerian edible mushroom, were carried out. A number of carbon compounds were utilised and glucose stimulated the best growth followed in order by mannose, cellulose and mannitol. Sorbose and myo-inositol enhanced the least growth. In the series of tested nitrogen sources, yeast extract was the most utilisable. Significant growth was supported by malt extract and L-tryptophan while poorest growth was recorded with sodium nitrate and ammonium sulphate. The best carbon/nitrogen ratio was 2:3 but the least utilised ratio was 5:1. Calcium and magnesium were the best macro-elements while micro-elements (copper and zinc) enhanced optimum growth.

1. Introduction

Psathyereilla atroumbonata (Pegler), is an edible mushroom which belongs to the phyllum basidiomycetes, order *agaricales* and family *coprinaceae* (Alexopolous, Mims & Blackwell, 1996; Makinen, 1977; Zoberi, 1972). This fungus is widely distributed in nature. It is found in tropical and subtropical regions of the world growing on forest litter, soil and dead wood (Pegler, 1977; Nicholson, 1996). *Psathyerella atroumbonata* is an excellent source of food among Yoruba people and many other tribes in Nigeria (Makinen, 1977; Oso, 1977a; Alofe, Odu & Illoh, 1998).

However, because of its unconspicuous basidiocarp, (Zoberi, 1972) people tend to ignore it. Those who know its value compete for it wherever it is growing wildly. Nigerian consumers of this edible fungus still depend on its seasonal occurrence, which is not regular. In spite of the food and potential medicinal value of some Nigerian mushrooms (Oso, 1977b) not much attention has been paid to their cultivation and commercial production. Therefore, the present study is aimed at providing useful preliminary information that could help in cultivation technology of *Psathyerella atroumbonata*.

2. Materials and methods

Fruit bodies of *Psathyerella atroumbonata* were collected from decaying wood of *Terminalia ivorensis* at the Ibadan University Botanical Gardens. Pure culture of the mycelia were obtained on potato dextrose agar (PDA) supplemented with 0.5% yeast extract. Nutrient requirements of this fungus were determined by the mycelia dry weight method (Fasidi & Jonathan, 1994). The basal medium was supplemented with different nutrients in 1000 cm^3 of de-ionised water inside the conical flask. Streptomycin sulphate (50 mg) was added to suppress bacterial growth and pH was adjusted to 6.5. The basal medium was dispensed into 250 cm^3 jam bottles (30 cm^3/bottle) and the mouth of each bottle was covered with aluminium foil. These were autoclaved at 1.02 kg cm^{-3} pressure at a temperature of 121°C for 15 min and each treatment had three replicates. Each bottle was inoculated with a 0.7 cm-diameter culture of *Psathyerella atroumbonata*.

Incubation was carried out for 7 days at 30±2°C, after which mycelia were harvested, oven-dried at 55°C for 18 h and weighed.

2.1. Effect of carbon compounds

The liquid medium used contained yeast extract, 2.5 g; KH_2PO_4, 0.05 g; MgSo4.$7H_2O$, 0.05 g; $FeSO_4$, 0.01 g; KNO_3, 1.55 g and 1000 cm^3 of de-ionised water. The liquid medium was supplemented separately with 1% carbon of each carbon compound. Complex carbon compounds were supplemented at the rate of 10 g/1000 cm^3. The medium without any carbon source served as

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the control (Kadiri & Fasidi, 1994; Chandra & Purkayastha, 1977).

2.2. Utilisation of nitrogen sources

The basal medium was made up of KH_2PO_4, 0.5 g; $MgSO_4$. $7H_2O$, 0.5 g; glucose, 10 g; thiamine hydrochloride, 0.5 mg and 1000 cm^3 of de-ionised water. Complex nitrogen sources (peptone, urea, yeast extract, malt extract and casein hydrolysate) were added separately at the rate of 2 g/1000 cm^3. The amount of nitrogen in amino acids and inorganic compounds was 0.1% nitrogen. The basal medium without nitrogen source served as the control.

2.3. Effect of different carbon/nitrogen ratios

The basal medium used was the same as for the nitrogen sources but, without glucose. The best carbon- and nitrogen sources in the last two experiments, i.e. glucose and yeast extract (0.1 g of each), were supplemented in the 1000 cm^3 of basal medium; this formed a ratio 1:1. Other ratios were also prepared to form different concentrations (Fasidi & Olorunmaiye, 1994).

2.4. Utilisation of macro-nutrients

The basal medium contained glucose, 10 g, $NaNO_3$, 2 g, $MgSO_4 \cdot 7H_2O$, 0.2 g, $CaCl_2$, 0.3 g, thiamine hydrochloride, 0.5 mg and 1000 cm^3 of de-ionised water. Each macro-element to be tested for was replaced by its ammonium conjugate (e.g. $NaNO_3$ was replaced by NH_4NO_3 and $MgSO_4$ was replaced by [$(NH_4)2\ SO_4$)]. Two sets of controls were used, one having all the macro-nutrients and the other having none.

2.5. Effect of trace elements

Five trace elements (copper, iron, manganese, cobalt and zinc) in their sulphate form, were added separately to the basal medium at the rate of 10 mg/1000 cm^3. Two sets of controls were also prepared. The first consisted of all the trace elements while the second set contained basal medium without any micro-element.

2.6. Analysis of data

All the data obtained from this investigation were analysed by analysis of variance and the tests of significance were determined by Duncan's multiple range tests.

3. Results and discussion

The results of the study carried out on carbon requirement of *Psathyerella atroumbonata* showed that glucose was the best carbon source (Table 1). This was closely followed by mannose and cellulose which were significantly different from each other ($P = 0.01$). The utilisation of glucose by some other tropical edible macro fungi has been reported (Fasidi & Olorunmaiye, 1994; Fasidi & Jonathan, 1994; Hong, 1978; Chandra & Purkayastha, 1977; Oso, 1977a). The preference of glucose over other carbon compounds may be due to the ease with which this sugar was metabolised to produce cellular energy (Garraway & Evans, 1984; Jandaik & Kapoor, 1976). Mannose, the second best carbohydrate is an isomer of glucose which can be transformed to glucose during metabolism (Morrison and Boyd, 1992). Griffin (1994) suggested that mannose and fructose are the most commonly utilised sugars after glucose. Myo-inositol, the least utilisable carbon source, is not known to have a significant effect on the growth of mushrooms. Its poor effect on the mycelial growth of *Psathyerella atroumbonata* may be due to the inability of this fungus to produce enzymes to metabolise this sugar alcohol.

Of all the tested nitrogen sources, the best mycelial yield was recorded in the medium that contained yeast extract (Table 2). This was followed by malt extract and L-tryptophan. This result is similar to that reported by Fasidi and Olorunmaiye (1994) on *Pleurotus*

Table 1
Utilisation of carbon compounds for growth by *Psathyerella atroumbonata* in liquid medium[a]

Carbon compounds	Mycelial dry weight (mg cm^{-3}; mean of three replicates)	Final pH of the filtrate
Monosaccharides		
Arabinose	90.0 ef	6.1
Fructose	110.0 d	5.7
Galactose	65.0 hi	5.9
Glucose	210.0 a	6.1
Mannose	173.0 b	6.2
Sorbose	50.0 jk	6.5
Rhamnose	60.0 ij	6.6
Oligosaccharides		
Cellobiose	60.0 ij	6.2
Lactose	60.0 ij*	6.3
Maltose	100.0 de	7.0
Raffinose	80.0 fg	6.0
Sucrose	65.0 hi	5.9
Polysaccharides		
Cellulose	163.3 bc	6.7
Dextrin	70.0 gh	6.5
Starch	85.0 fg	6.8
Sugar alcohols		
Mannitol	153.3 c	5.9
Myo-inositol	50.0 jk	6.3
Control	35.0 1	7.0

[a] Column values followed by the same letters are not significantly different by Ducan's multiple range test ($P = 0.01$).

Table 2
Utilisation of different nitrogen compounds for growth by *Psathyerella atroumbonata* in liquid medium[a]

Nitrogen compounds	Mycelial dry weight (mg cm^{-3}; mean of three replicates)	Final pH of the filtrate
Inorganic sources		
Ammonium nitrate	63.3 fg	6.4
Ammonium sulphate	36.7 h	6.7
Calcium nitrate	70.0 ef	5.8
Potassium nitrate	46.7 gh	6.2
Sodium nitrate	36.7 h	6.7
Amino acids		
L-Aspartic acid	100.0 d	6.3
L-Asparagine	70.0 ef	5.9
D-Alanine	60.0 fg	5.5
L-Glutamic acid	60.0 fg	6.2
L-Glutamine	80.0 e	6.1
D-Cysteine	70.0 ef	6.9
DL-methionine	63.3 fg	7.0
L-Tryptophan	120.0 bc	7.3
DL-phenyl alanine	100.0 d	6.5
DL-leucine	40.0 h	6.7
L-lysine	80.0 e	6.1
Complex organic sources		
Casein	80.0 e	7.3
Malt extract	130.0 b	6.1
Peptone	90.0 de	6.4
Urea	70.0 ef	6.7
Yeast extract	170.0 a	5.9
Control	40.0 h	6.2

[a] Column values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$).

Table 3
Utilisation of different carbon/nitrogen ratios for growth by *Psathyerella atroumbonata* in liquid medium[a]

Carbon/nitrogen ratio	Mycelial dry weight (mg cm^{-3}; mean of three replicates)	Final pH of the filtrate
1:1	70.0 de	6.8
1:2	150.0 ab	6.4
1:3	100.0 d	6.6
1:4	86.7 de	6.2
1:5	73.3 de	6.7
2:1	133.3 bc	7.3
2:3	176.7 a	6.1
2:5	133.3 bc	60
3:1	100.0 cd	5.9
3:2	110.0 cd	6.4
3:4	153.3 ab	6.7
3:5	130.0 bc	6.2
4:1	90.0 de	6.3
4:3	100.0 d	5.8
4:5	130.0 bc	6.0
5:1	56.7 fg	6.7
5:2	63.3 ef	6.4
5:3	90.0 de	6.3
5:4	100.0 d	5.8
0:0 (Control)	43.0 h	7.1

[a] Column values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$).

tuber-regium and Alberghina (1973) on *Neurospora crassa*. The stimulatory effect of these two compounds on the growth of *Psathyerella atroumbonata* may be attributed to their carbohydrate and protein compositions (Alberghina, 1973; Bolton & Blair, 1982).

Amino acids generally supported good growth of this macro fungus with L-tryptophan stimulating the best yield (120 mg cm^{-3}; Table 2) followed in order by L-aspartic acid and DL-phenyl alanine. Madunagu (1988) obtained a similar result with *Pleurotus squarrosulus*. With the exception of calcium nitrate and ammonium nitrate, which supported moderate growth of 70 and 63 mg cm^{-3}, respectively; all other inorganic nitrogen sources enhanced poor growth. Ammonium sulphate and sodium nitrate supported growth of 36.7 mg cm^{-3} which is lower than that of the control. Nitrate ions have been implicated in the inhibitory effect of some basidiomycetes (Griffin, 1994). Sulphate ion (SO_4^{2-}) is a large radical (Garraway & Evans, 1984) which may be difficult to transport across the fungal membrane where it can promote growth.

All the carbon/nitrogen ratios used in this study promoted growth significantly (Table 3) ($P = 0.01$). The fungus grew best on a medium with ratio 2:3 followed by 3:4 while the least growth was obtained with the ratio 5:1. These ratios were different from that obtained by Chandra and Purkayastha (1977; for *Agaricus campestris*) and Fasidi and Olorunmaiye (1994; for *Pleurotus tuber-regium*). This shows that *Psathyerella atroumbonata* has specific growth requirements. This fungus is able to utilise substrates that contain carbon and nitrogen sources within a tolerable limit (Jandaik & Kapoor, 1976).

Growth of *Psathyerella atroumbonata* was significantly enhanced in the medium that contained all the macro-elements (complete medium; $P = 0.01$). This suggests that all the macro-elements used are required for the growth of this fungus although the level of utilisation varies (Table 4). Complete medium. without Na, stimulated the best growth followed in order by medium that lacks potassium, calcium and magnesium. This result indicates that calcium is the most utilisable macro-element, followed by magnesium, potassium and sodium. Similar observations were made by Fasidi and Jonathan (1994) on *Volvariella esculenta*. Griffin (1994) attributed the importance of calcium in fungal growth to its enzyme activity while magnesium is important in ATP metabolism. Poorest growth by sodium supports the suggestion of Sykes and Porter (1973) that only fungi in marine habitat needed sodium for the growth and metabolism.

Table 4
Utilisation of different macro-elements for growth by *Psathyerella atroumbonata* in liquid medium[a]

Macro-elements	Mycelial dry weight (mg cm^{-3}; mean of three replicates)	Final pH of the filtrate
Complete medium (Control 1)	120.0 a	6.7
Complete medium without magnesium	73.3 c	6.3
Complete medium without calcium	60.0 c	6.5
Complete medium without potassium	85.0 bc	6.3
Complete medium without sodium	106.7 ab	5.9
Basal medium only (Control 2)	43.3 d	5.8

[a] Column values followed by the same letters are not significantly different by Duncan's multiple range test ($P=0.01$).

Table 5
Utilisation of trace elements for growth by *Psathyerella atroumbonata* in liquid medium[a]

Trace elements	Mycelial dry weight (mg cm^{-3}; means of replicates)	Final pH off filtrate
Complete medium (Control 1)	100.0 b	6.7
Complete medium without copper	70.0 c	5.9
Complete medium without iron	93.3 b	6.4
Complete medium without manganese	106.7 ab	6.6
Complete medium without cobalt	126.7 a	6.0
Complete medium without zinc	76.7 c	6.3
Basal medium only (Control 2)	40.0 d	5.8

[a] Column values followed by the same letters are not significantly different by Duncan's multiple range test ($P=0.01$).

Among the trace elements, the highest mycelial yield was obtained on Co-free medium followed in order by media without maganese, iron, zinc and copper (Table 5). This result implies that *Psathyerella atroumbonata* can grow effectively in the absence of cobalt and maganese. Inability of this fungus to utilise these two trace elements for growth may be related to their toxicity to fungal cells. A similar toxic effect was reported by Humfeld and Sugihara (1952; for *Agaricus campestris*) and Chandra and Purkayastha (1977; for *Volvariella volvacea*). The least growth recorded, on copper- and zinc-free media, implies that they were needed for growth. Although these two trace elements have different values, they are not different from each other statistically ($P=0.01$). Both copper and zinc are needed for fungal enzyme activities but, in addition, zinc is needed for intermediary metabolism (Griffin, 1994). Poor mycelial yield, obtained on basal medium (Control 2), suggests that *Psathyerella atroumbonata* requires some trace elements for its growth (Griffin, 1994; Garraway & Evans, 1984).

It is clearly shown from these studies that growth of *Psathyerella atroumbonata* is enhanced by glucose and yeast extract. These could be incorporated into the medium in a ratio of 2:3. Calcium, magnesium and potassium also stimulated good growth, while copper and zinc (in very low concentration) are needed for mycelial propagation of *Psathyerella atroumbonata*. All these can be supplemented in the growth media to produce high mycelial yield needed for spawning and fruit body production of this excellent edible fungus.

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