Influence of Environmental and Nutritional Factors on The Vegetative Growth of *Pleurotus pulmonarius*

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Abstract: *Pleurotus pulmonarius* is an edible macrofungus that is produced commercially. Cultural studies to determine the most suitable culture media for the vegetative growth was carried out. The effect of environmental and nutritional factors was also investigated. Temperature ranging between 10-25°C and pH values (4.0-8.0) influences was determined on different chemically defined medium (potato dextrose agar, malt extract agar and yeast extract agar). Basal medium was prepared for the nutritional sources (carbon, nitrogen, minerals and vitamins) and all were incubated at 30 ± 2°C for 7days. The mycelia were recovered by sterile filtering and drying. The highest mycelia growth rate was obtained between pH 6.0-8.0 and at 25°C. Fructose was the most utilized carbon source, L-leucine the most utilized nitrogen source and Vitamin C most utilized vitamin source. The most utilized macroelements was the combination of magnessium and calcium while for the microelements the best combination was zinc and copper. These requirements are suitable for the optimal production of active mycelium of *Pleurotus pulmonarius.*

Keywords: carbon, mycelia, nitrogen, pH, *Pleurotus pulmonarius* and vitamins.

Introduction

Pleurotus pulmonarius is an edible white rot fungi (WRF) commonly known as the Phoenix Mushroom, or the Lung Oyster. *Pleurotus pulmonarius* grows on stumps and trunks of a wide range of deciduous trees, usually in the form of overlapped leafs (Wasser and Weis, 1999). *Pleurotus pulmonarius* is a potential protein source especially in developing countries where animal protein is scarce and expensive (Quimio *et al*., 1990; Jonathan *et al*, 2012a).

In the traditional area, extracts from *Pleurotus* species have been reported to be used in curing some ailments (Osemwegie *et al*., 2010; Idu *et al*., 2007). Many works have reported the requirements for the vegetative growth of some Nigerian mushrooms; *Pleurotus florida* (Adenipekun and Gbolagade, 2006), *Tricholoma lobayensis* (Jonathan and Fasidi, 2003), *Pleurotus ostreatus* (Nwokoye *et al*., 2010) while little work have being done on *P. pulmonarius*. Therefore, the aim of this work was to evaluate the influence of the nutritional and environmental factors on the mycelia growth of this edible fungus.

Materials and Methods

Source of organism: The spawn of the fungus was obtained from Mycology/Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Oyo State, Nigeria.

Effect of temperature and pH on vegetative growth of *P. pulmonarius:* The mycelium was obtained by growing the spawn on malt extract agar: the agar had already been prepared according to manufacturer's instruction and pouted in a petri dish to solidify.

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To investigate the effect of pH on growth, the culture medium (malt extract agar) was adjusted to pH values between 4.0-8.0 using 1M NaOH and 0.1M HCl and autoclaved at 121°C for 15 minutes. After cooling, the medium was dispensed into sterile petri dishes and each plate was inoculated with mycelia from a 7 day old culture. The mycelium from the 7 day old culture was collected using a cork borer of 7 mm in diameter. This was then incubated at 25°C. The growth was monitored for 7days, and measurement taken with a metre rule.

The same method was employed for temperature except that the plates were incubated at (10, 15, 20, 25°C). The temperature was maintained in a regulated incubator.

Determination of carbon requirement for mycelia growth of *P. pulmonarius*

The basal medium consisted of peptone (2.5g), KH_2PO_4 (0.25g), $MgSO_4.7H_20$ (0.25g) in 500ml of distilled water. The medium was supplemented separately with 10.0g/l of each carbon compound (glucose, fructose, lactose and maltose). For polysaccharides (starch) the medium was supplemented with 5.0g/l. The control medium lacked all these carbon compounds. Mycelia from a 7 day old culture was punched into the 250ml bottle and incubated at room temperature for 7days. The mycelia were recovered by sterile filtering and drying to a constant weight (Jonathan and Fasidi, 2003).

Determination of nitrogen requirement for mycelia growth of *P. pulmonarius*

The basal medium used contained glucose (5.0g), NaCl (0.05g), $CaCl_2$ (0.05g), KH_2PO_4 (0.25g), $MgSO_4.7H_20$ (0.25g) and ascorbic acid (250µg) in 500ml of distilled water. The medium was supplemented separately with inorganic compounds ($NaNO_3$ and NH_4NO_3; 0.5g/l); amino acid (L-leucine and L-asparagine; 0.5g/l) and complex organic

compounds (peptone and urea; 1.0g/l). Mycelia from a 7-day old culture was punched into the 250ml bottle and incubated at room temperature for 7days. The mycelia were recovered by sterile filtering and drying to a constant weight (Jonathan and Fasidi, 2003).

Determination of vitamins requirement for mycelia growth of *P. pulmonarius*

The basal liquid medium used was made up of glucose (5.0g), peptone (1.0g), KH_2PO_4 (0.25g) and $MgSO_4.7H_2O$ (0.25g) in 500ml of distilled water. Vitamins used include folic acid (B9), pyridoxine (B6), riboflavin (B2) and ascorbic acid (C). The vitamins were added separately to give a concentration of 250µg per 500ml. Two control setups were made: one with all the vitamins and the other without vitamins. Mycelia from a 7day old culture was punched into the 250ml bottle and incubated at room temperature for 7 days. Mycelia were recovered by sterile filtering and drying to a constant weight (Jonathan and Fasidi, 2003).

Determination of mineral elements requirement for mycelia growth of *P. pulmonarius*

The basal medium used was made up of maltose (10g), ammonium nitrate (NH_4NO_3, 2g), ammonium chloride (NH_4Cl, 0.3g), ammonium sulphate $(NH_4)^2SO_4$ 0.2g) and pyridoxine vitamin B6 (0.5g). Four control setups were made: one contained all macro elements; another contained all trace elements while the other two contained the basal medium only. Mycelia from a 7day old culture was punched into the 250ml bottle and incubated at room temperature for 7 days. Mycelia were recovered by sterile filtering and drying to a constant weight.

Statistical analysis

Data obtained from these studies were analyzed using ANOVA and test of significance was determined by Duncan's multiple range test.

Results and Discussion

The highest mycelia growth was seen on malt extract agar at pH 6.0 and 8.0 (Figure 1). The highest mycelia growth rate was seen on MEA at 25°C which had the highest diameter for the growth compared with other culture medium tested (potato dextrose agar and yeast extract agar). This agrees with Quimio (2001) who gave the optimum temperature for *P.pulmonarius* to fruit faster as 25°C (Figure 2). The five carbon sources used in this study supported the mycelia growth of *P.pulmonarius* (Table 1).

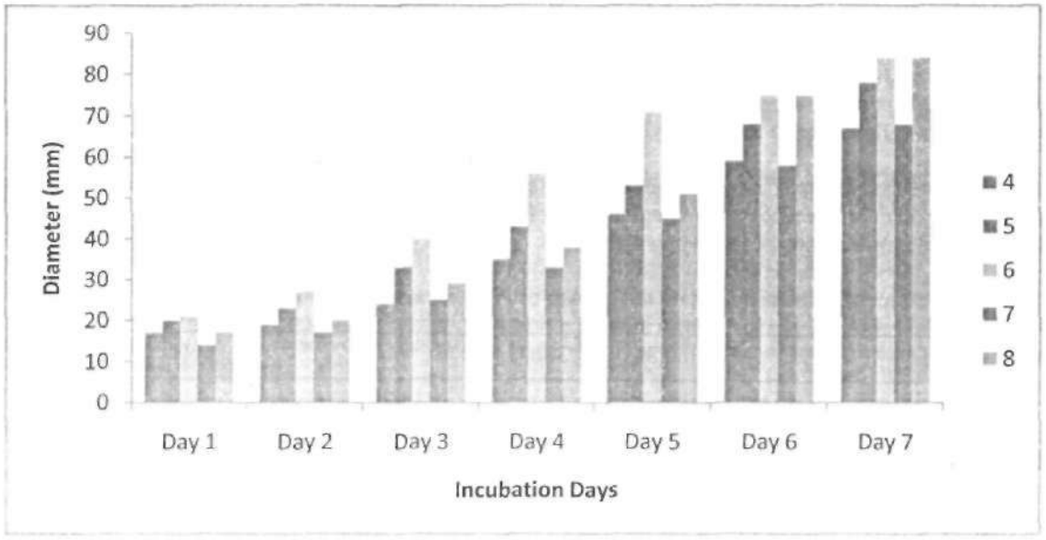

Figure 1: Effect of pH on the mycelia growth of *P. pulmonarius* on MEA

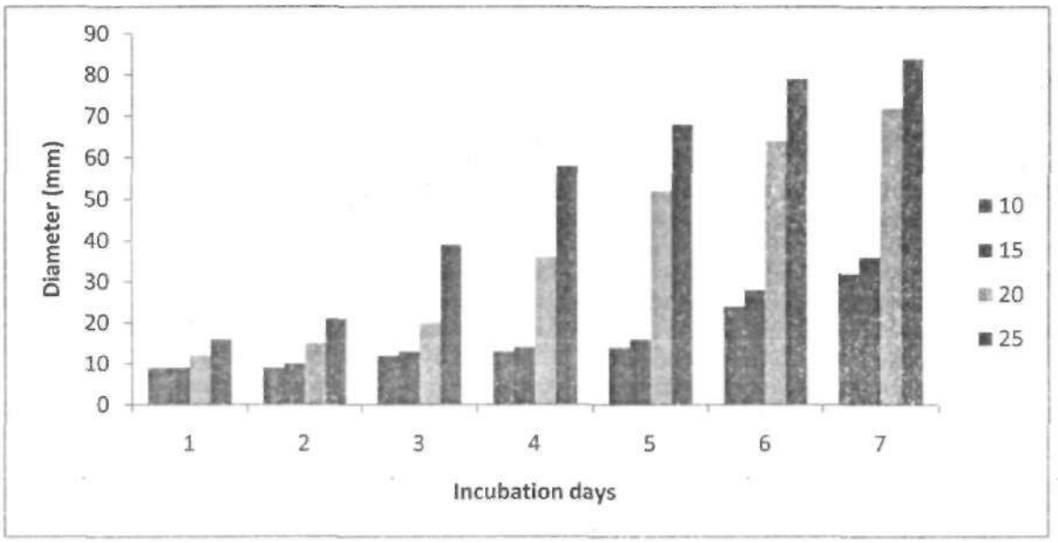

Figure 2: Effect of temperature on the mycelia growth of *P. pulmonarius* on MEA

The most utilized carbon source was fructose followed by maltose, starch, lactose and glucose (Table 1). This result is similar to that obtained by Jonathan and Fasidi (2003) for *Tricholoma lobayensis* which had the best carbon sources as mannitol, fructose and glucose. The utilization of fructose may be due to the fact that it is found in many plants and easily broken down. A work on *Pleurotus ostreatus* by Nwokoye et al (2010) declared glucose followed by starch and maltose as the most stimulatory carbon source for the mycelia growth.

Of all the tested nitrogen sources, L-leucine gave the best mycelia yield followed by L-asparagine, sodium nitrate, ammonium nitrate and peptone in which there was significant difference (Table II) among them; whereas the work of Jonathan and Fasidi (2003) on *Tricholoma lobayensis* (Heim) declared ammonium nitrate as the most stimulatory nitrogen source. A similar work on *Pleurotus ostreatus* (Fr.) Singer by Nwokoye *et al* (2010) gave peptone as the best nitrogen source for the mycelia growth. Asparagine was the most utilised nitrogen source for *Lepiota procera* (Jonathan and Fasidi, 2003). The ability of this amino acid to give the best mycelia yield may be due to its role in the production of growth hormones. It was observed from the result that *P. pulmonarius* prefers simple amino acids for its mycelia yield.

Table 1: Effect of carbon sources on the growth of *P. pulmonarius* in submerged medium

Carbon Sources	Mycelial dwt (mg/30ml)	Final pH
Lactose	344.7^{ba}	6.54
Maltose	552.0^{a}	6.59
Glucose	308.0^{ba}	6.33
Fructose	587.5^{a}	5.35
Starch	366.6^{ba}	7.15
Control	104.0^{b}	6.87

Key: dwt=dry weight; Mycelial dwt are mean of 3 replicates run.

Mean values followed by the same letter(s) are not significantly different ($P \leq 0.05$) by Duncan's Multiple Range test

Table 3 reveals that the mycelium which ought to increase with the combination of all the vitamins was not really high. This suggests that not all the vitamins used are needed for the mycelia growth of the fungus. The control medium (addition of all vitamins) and Vitamin B2 were significantly different from all other vitamins used whereas Adenipekun and Gbolagade (2006)'s work on *Pleurotus florida* declared no significant difference in the mean values of Vitamin C and Vitamin B2. The organism could not utilize the Vitamin B2 added because the organism is able to synthesize the vitamin itself and might be toxic to the organism when added in excess.

Table II: Effect of nitrogen sources on the growth of *P. pulmonarius* in submerged medium

Nitrogen Sources	Mycelial dwt (mg/30ml)	Final pH
NH_4NO_3	51.3^b	5.97
$NaNO_3$	64.3^b	6.04
L-Asparagine	290.8^a	5.46
L-Leucine	323.3^a	5.33
Urea	89.5^b	5.78
Peptone	45.0^b	4.16
Control	27.0^b	6.62

Key: dwt=dry weight; Mycelial dwt are mean of 3 replicates run. Mean values followed by the same letter(s) are not significantly different ($P \leq 0.05$) by Duncan's Multiple Range test

Table III: Effect of vitamin sources on the growth of *P.pulmonarius* in submerged medium

Vitamin Sources	Mycelial dwt (mg/30ml)	Final pH
Vitamin C	129.9^a	5.86
Vitamin B2	58.6^b	5.65
Vitamin B6	113.9^a	5.44
Vitamin B9	114.7^a	5.42
Control + vitamins	90.8^b	5.69
Control- vitamins	105.2^a	6.00

Key: dwt=dry weight; Mycelial dwt are mean of 3 replicates run. Mean values followed by the same letter(s) are not significantly different ($P \leq 0.05$) by Duncan's Multiple Range test

Table 4 shows the effects of mineral elements on the growth of the test organism, the best growth was observed in the complete medium without Sodium (Na) while the least was recorded in the complete medium containing all the macro elements. There was significant difference among the values obtained for the macro elements. Similar observations were made by Jonathan and Fasidi (2003) for *Tricholoma lobayensis* (Heim) whose best growth was recorded in a Na-free medium.

For the microelements, the highest mycelia yield was obtained in medium without Iron (Fe) while the least was observed in medium without copper (Cu). There was also significant difference among the tested microelements. Adenipekun and Gbolagade (2006) reported that the complete medium without Iron gave a poor growth followed closely by the Zinc (Zn) free medium.

Table IV: Effect of different mineral elements on the growth of *P.pulmonarius* in submerged medium.

Mineral Elements	Mycelial dwt (mg/30ml)	Final pH
Macro elements		
Basal medium (control 1)	201.9[a]	5.71
Complete medium without Mg	196.1[a]	5.54
Complete medium without Ca	266.7[a]	5.08
Complete medium without Na	277.7[a]	5.60
Complete medium (control 2)	70.2[b]	5.73
Microelements		
Basal medium (control 1)	232.6[a]	5.71
Complete medium without Fe	279.0[a]	4.82
Complete medium without Zn	153.6[b]	4.81
Complete medium without Cu	153.0[b]	4.67
Complete medium (control 2)	240.6[a]	5.75

Key: dwt=dry weight; Mycelial dwt are mean of 3 replicates run.
Mean values followed by the same letter(s) are not significantly different ($P \leq 0.05$) by Duncan's Multiple Range test.

Conclusion

From this study, the needed requirements to obtain good mycelia yield include pH 6.0-8.0 and temperature at 25°C. The most suitable culture medium for mycelia growth is malt extract agar. The macrofungi was able to utilize fructose as the carbon source and L-leucine as the nitrogen source. The vitamin utilized by the macro fungi was Vitamin C and mineral elements enhancing good mycelia yield include combination of magnesium and calcium (macro elements) and combination of zinc and copper as the trace elements. These findings are essential for production of active mycelium of *Pleurotus pulmonarius* which will be used in spawning and good fruit body production of the fungus. More growth rate will be obtained if carbon and nitrogen is added together in varying ratios.

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