Studies on Mycelial Growth Requirements of Pleurotus Ostreatus (Fr.) Singer

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Abstract-- Studies were carried out on the growth requirements of Pleurotus ostreatus (oyster mushroom), an edible mushroom. This organism was able to grow optimally at a temperature of 28°C and pH of 9. It utilized various carbohydrate sources such as glucose, fructose, maltose, ethanol, starch, sucrose and lactose, with glucose the most utilized and this significantly enhanced mycelial growth. The least utilized carbohydrate was lactose. Peptone supported the greatest mycelial growth as a nitrogen source followed by yeast extract while inorganic nitrogen sources had no appreciable effect. Evaluation of agro industrial wastes like corn cob, cotton waste, paperwaste and sawdusts of Mansonia altissima and Irvingia as to their suitability for mushroom production gave corn cob as the best substrate for the mycelia growth of the organism.

Index Term-- Growth requirements : mycelia : agro- wastes : substrates.

I. INTRODUCTION

The common name “Oyster mushroom” refers to several species of edible mushroom belonging to the genus Pleurotus. In Nigeria, the most prized edible species are Pleurotus, Termitomyces, Tricholoma and Volvariella [1]. Mushroom cultivation serves as the most efficient and economically viable biotechnology for the conversion of lignocellulose waste materials into high quality protein food and this will naturally open up new job opportunities especially in rural areas [2;3]. Edible mushrooms like Agaricus, Volvariella and Pleurotus ostreatus are commercially produced and sold in markets in Asia, America and Europe. In Nigeria, indigenous mushroom are still hunted for in forests and farmlands for sale. The need for commercial production of all edible mushrooms in Nigeria cannot be over emphasized in view of its potential contribution to agricultural production and as a source of cheap protein. Mushrooms are cultivated on various waste products of human, agricultural, forestry and industrial concerns and effectively utilize these wastes, thus, the growth of the fungi on these substrates help to prevent environmental and health hazards posed by indiscriminate dumping of these materials [4]. Mushrooms are highly nutritious and are important features of human diet worldwide. High protein content of as much as 50 to 84% dry matter has been detected in the fruit bodies and mycelia of P. ostreatus, Lentinus edodes, Volvariella esculenta & Termitomyces clypeatus. [5, 6 and 7]. Their mycelia also contain amino acids like glycine, valine, threonine, serine, leucine, proline, methionine, asparagine, glutamine, lysine, arginine, histidine, cysteine and alanine [8].

Thus, the objectives of this study include: To determine the optimal temperature and pH necessary for mycelial growth of P. ostreatus, to study the effects of different carbon and nitrogen sources on the mycelial growth of P. ostreatus and to evaluate the suitability of agro-industrial wastes like banana leaves, sawdusts of Mansonia and Irvingia, cotton waste, grass & paper waste as substrates for the cultivation of P. ostreatus.

II. MATERIALS AND METHODS

Sources of materials
The primary inoculum for this study was obtained from the spawn of P. ostreatus collected from Zartech Company, Ibadan, Oyo State, Nigeria.

To determine optimal temperature for growth of P. ostreatus
The temperature requirement for the optimal growth of this organism was investigated by incubating a 5mm disc of a 4 day old pure mycelia of P. ostreatus on PDA. This treatment was replicated thrice and the organism was incubated at temperature range of 18-47°C for 6 days. Mycelial diameter was measured with a meter rule to estimate the growth [7].

pH requirement for the growth of P. ostreatus
The basal medium in one litre of distilled water consisted of Ferrous sulphate FeSO₄ (0.01g), Magnesium sulphate MgSO₄ (0.05g), K₂HPO₄ (0.05g), KH₂PO₄ (0.03g), Yeast extract (2.50g) Glucose (10g) CuSO₄ (0.15g) 1M NaOH, 1M HCL, Streptomycin (0.1g). The pH was adjusted within the range of 3 – 10 using 1M HCl and 1M NaOH. Inoculation was done with a 5mm disc of a 4 day old mycelia of P. ostreatus and the beakers were incubated for 6 days at 28°C. The mycelia mat in each beaker was filtered through a pre-weighed filter paper, dried at 80°C in an hot-air oven for 24hrs, cooled in a desiccator and weighed [9].

To determine the carbon requirement for mycelial growth of P. ostreatus Carbohydrate Sources
The basal medium consisted of peptone (2kg), KH₂PO₄ (0.5g), MgSO₄ (0.5g), Streptomycin (0.1g) and distilled water up to 1000ml and supplemented with carbon sources; maltose, fructose, glucose, lactose, sucrose, starch and ethanol 1% (w/v). The pH of the basal medium was adjusted to pH 9 with 1M NaOH. These were then sterilized at 1.05kg/cm² pressure for 15 minutes and growth of the organism was assessed using the dry weight method.

Growth on different concentration of glucose source
For P. ostreatus, glucose proved to be the best carbon source. Different concentrations of glucose 1-10g were dissolved in 100ml of the basal medium at pH of 9.0.
To determine the nitrogen requirement for mycelial growth of *P. ostreatus*

The basal medium consisted of glucose (9g), KH$_2$PO$_4$ (0.5g), MgSO$_4$$\cdot$7H$_2$O (0.5g), streptomycin (0.1g) dissolved in 1 litre of distilled water. The nitrogen sources utilized were NaNO$_3$, KNO$_3$, NH$_4$ (SO$_4$)$_2$, peptone, urea, and yeast. The amount of nitrogen in each supplementary compound was equivalent to that in 2g NaNO$_3$.

**Growth on different concentrations of the nitrogen source**

The basal medium consisted of glucose (9g), KH$_2$PO$_4$, MgSO$_4$$\cdot$7H$_2$O, peptone, streptomycin (0.1g) and distilled water 1 l. For *P. ostreatus*, peptone proved to be the best nitrogen source hence different concentrations of peptone, 1-10g were dissolved in 100ml of the basal medium.

**The Control**

In all cases, the basal medium without any inoculum of *P. ostreatus* were used as controls.

**Evaluation of agro-industrial wastes as substrates for the growth of *P. ostreatus***

Cotton waste (Gossypium spp) sawdusts of *Mansonia altissima* and *Irvingia*, banana leaves (*Ficus macellenlandii*), elephant grass (*Andropogon* spp), corn cob (*Zea mays*) and paper waste were evaluated as substrates for the growth of *P. ostreatus*.

200g of the sawdusts *Mansonia* and *Irvingia* were weighed, mixed with 2% CaCO$_3$ suspension; the excess water was expelled from the substrates by pressing hard and the materials were composted for a period of 5 weeks, while, grass (*Andropogon* spp), banana leaves (*Ficus macellenlandii*), corn cob (*Zea mays*) and cotton waste (Gossypium spp) were composted for 4 days. They were then put into 10cm (diameter) Petri dishes. Dishes were sterilized at 121°C for 15 minutes, cooled and inoculated with a 4 day old mycelia (5mm in diameter) of *P. ostreatus*. These were incubated at 28°C for 8 days. Growth was measured using a meter rule [6].

**Determination of moisture content of the substrates**

The moisture content of the agro wastes was determined using the methods of Staples [10].

**Statistical Analysis**

In all cases the samples were in three replicates and their means were quoted with standard errors (SE). Statistical analyses were carried out using Analysis of variance 1 at 5 and 1% levels [11].

**III. RESULTS AND DISCUSSION**

The mycelium of *P. ostreatus* grew optimally at a temperature of 28°C and pH of 9 (fig 1 and 2). Thus the ability of the mycelia to tolerate this temperature and pH range of 3-10 enabled them to flourish in agro wastes in the tropics [12]. The temperature obtained was not in agreement with that reported by Leeward, [13] for *P. ostreatus* (10-20°C), thus inferring that the strain used for this study was different from that mentioned above. [14] mentioned that there were three strains of *P. ostreatus*, the high temperature, medium and low temperature strains in the range of 25°C-30°C, 16°C-22°C and 12°C-15°C, respectively, inferring that the strain used for this study belonged to the high temperature strain, while Fasidi, [6] reported an optimum pH of 6.0 for *Volvariella* esculenta and [7] found that 35°C and 7 were optimal temperature and pH values for mycelial growth of *V. volvacea*. [15] reported that optimum pH of *V. volvacea* varies from strain to strain as strain CT 13 had an optimum pH of 5 and another strain, a pH of 6.6. This further confirms that the strain used for this study is different from that of [13]. High pH tends to suppress the growth as well as antagonize certain weed fungi in compost thus reducing competition for the mushroom [5]. [16] established that the spread of mycelial growth of *Pleurotus spp* was related to the temperature of the substrate. Mycelial development forms the vegetative growth phase of mushroom growing and temperature is highly important since it affects the growth and adaptability as well as the quantity of the quality of fruiting-bodies produced [17].

Glucose was found to be the best carbon source for *Pleurotus ostreatus* followed by maltose and starch (fig. 3). [18] found that raffinose stimulated the highest mycelial dry weight in *Pleurotus tuber-regium*, followed by fructose and glucose. The preference of glucose over other carbon source by *Pleurotus ostreatus* may be due to the ease with which it was metabolized to produce cellular energy for the growth of the organism. [19] mentioned that the ability of *Pleurotus spp* to use different carbon sources may be an expression of the physiological differences in the species or of the isolates, since other isolates of the same species might give different results. Peptone significantly increased the growth of *Pleurotus ostreatus* (fig 5) while inorganic nitrogen sources had no appreciable effect. This implies that the fungus had greater preference for peptone and yeast extract than the inorganic compounds such as sodium nitrate (NaNO$_3$) and ammonium sulphate (NH$_4$)$_2$SO$_4$. The organism grew optimally on 5% peptone suggesting that there is an optimal concentration for nitrogen to obtain maximum yield of *Pleurotus ostreatus* (Fig 6).

Evaluation of agro industrial waste suitable for mushroom production gave corn cob as the best substrate for the mycelia growth of *Pleurotus ostreatus* (Fig. 7). This may be due to the saprophytic ability of this fungus to produce extracellular enzyme hydrolyzing enzymes such as cellulose, lignase and laccase to hydrolyse cellulose, hemicelluloses and lignin present in these substrates to simple sugars for growth [20]. Shah et al, [21] reported that sawdust gave maximum yield of *Pleurotus ostreatus* and the least was in the leaves, while [22] reported substrates like corn cob, rice straw cotton waste, cocoyam peels and sawdusts of *Khaya ivorensis, Mansonia altissima, and Boscia angustifolia* supported the growth of *Pleurotus tuber-regium*. The moisture content of the substrates varied from 26% -67% showing that the agro wastes had different absorptive properties and this also affects the mycelial yield produced in each substrate (Fig 7). Successful utilization of agro-wastes for both mycelial and fruitbody formation of mushroom supply nutrients needed by this fungus to convert them to protein-rich palatable foods.
IV. CONCLUSION
Thus it can be concluded that maximal mycelial growth of *Pleurotus ostreatus* can be achieved by culturing the fungus at temperature of 28°C and pH of 9.0. The mycelial mass can also be enriched by growing in a medium containing 9% glucose and 5% peptone as carbon and nitrogen sources respectively and using corn cob as a substrate will give maximal mycelial extension of *Pleurotus ostreatus* which leads ultimately to produce fruitbodies of high yield. In addition, utilization agro wastes helps in reducing the mountain range of wastes, converting them into mushroom protein and vitamins. This represents one of the world’s untapped resources of tasteful food in the future and because mushroom cultivation needs only a small space, land can be conserved. Thus the use of wastes can provide more food, more jobs, better family income, and improved living standard, curb global warming and clear up the crop residues on road sides and forest margins [4].

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REFERENCES
[7] O. O. Kuforiji and I.O. Fasidi, Vegetative Growth requirements of *V. volvacea* a
   a. of Ibadan, 1990.
   a. pp 193.
   a. 1995, 204-252.
[14] S. T. Chang and T. H. Miles, Mushrooms: Cultivation, Nutritional value,
[15] D. S. Tseng, Studies on the nutritional requirement and improvement
[16] F. Zadrazil, Cultivation of *Pleurotus*. In the physiology and cultivation of edible
[17] O.O Kuforiji and I.O. Fasidi, Biodegradation of agro-industrial wastes by an
[18] O. O. Kuforiji and I. O. Fasidi, Growth of *P tuber-regium* a Nigerian
Fig. 1. Effect of temperature on mycelial growth of *P. ostreatus*.

Fig. 2. pH requirement of *P. ostreatus*. 

Dry weight of fungal mat (g/100ml) vs pH of the medium.
Fig. 3. Carbon requirement of *P. ostreatus*

Fig. 4. Effect of different concentrations of carbon on mycelial growth of *P. ostreatus*
Fig. 5. Nitrogen requirement of *P. ostreatus*.

Fig. 6. Mycelial growth of *P. ostreatus* in different concentrations of peptone.
Fig. 7. Evaluation of agro-wastes as substrates for the cultivation of *P. ostreatus*