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Physiological studies on *Schisophyllum commune* (Fr. Ex. Fr.) A Nigerian edible mushroom

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ABSTRACT: Studies were conducted to evaluate carbon and nitrogen sources required for the vegetative growth of *Schizophyllum commune*, a Nigerian edible mushroom. This fungus grew within a temperature range of 10 and 40°C (Optimum = 25°C) and pH range of4.0 and 8.5 (Optimum = 6.5). *S. commune* utilised a wide range of carbon and nitrogen compounds for its growth. The most stimulatory carbohydrate source was galactose, followed by mannitol and glucose while the least growth was supported by dextrin. All the organic and inorganic nitrogen compounds investigated significantly improved growth with casein hydrolysate being the best and sodium nitrate being the least. Likewise, carbon/nitrogen ratios affected the growth of this fungus and ratio 5:2 was the most stimulatory, while ratio 1:5 was inhibitory to growth. The significance of these findings was discussed.

Key words: *Schizophyllum commune*, carbon and nitrogen sources, stimulatory.

Introduction

Schizophyllum commune (Fr. Ex. Fr.) is one of the most common tropical macro-fungi (Zoberi, 1972; Singer, 1986). This edible mushroom which belongs to the phylum basidiomycota, order aphyllophorales and family schizophyllaceae is known locally as ‘ese adie’among the Yoruba people of South-Western Nigeria (Oso, 1981; Raper, 1983). The fruit bodies are xerophytic and are generally encountered growing on wood or other dead or living organic matter during the rainy season (Wessel, 1987). This white spored bracket fungi with sessile cap is an active destroyer of wood.

Schizophyllum commune is not very popular in the mushroom growing industries probably because of the small nature of its sporocarp and leathery consistency of matured fruit bodies. However, this organism is worthy of attention, if it is harvested young, it is an appetising delicacy with good flavour and medicinal value (Oso, 1981; Wessel, 1987; Stamets, 1993).

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Jonathan and Fasidi (2001a), reported that this higher fungus required exogenous supply of vitamins such as pyridoxine and folic acid for its vegetative growth. They also recorded significant growth of this fungus when mineral elements and phytohormone such as magnesium, potassium and 2,4 dichlorophenoxy acetic acid were incorporated into the growth medium. Therefore, the objectives of this present investigation is to provide more information on growth requirements of this fungus so as to make its cultivation a profitable venture.

Materials and Methods

Preparation of Mycelial Starter Culture

The sporophores of *Schizophyllum commune* were collected from the dead logs of *Ceiba pentrada* at the nursery section of Botany and Microbiology Department, University of Ibadan, Ibadan. The fruit bodies of this fungus were tissue cultured to obtain pure mycelia culture which were maintained on potato dextrose agar (PDA) supplemented with 0.5% yeast extract.

Effect of Temperature and pH on Mycelial Growth

The effect of temperature and pH on growth were determined by mycelia dry weight method (Fasidi, 1996). The basal liquid medium used consisted of $FeSO_4$ (0.01g); $MgSO_4.7H_2O$ (0.5g); KH_2PO_4 (0.05g); glucose (10.0g); mycological peptone (2.50g); KNO_3 (1.55g) and $10cm^3$ of micronutrients made up to $1000cm^3$ with de-ionised water. The component of the micronutrient solution in $1000cm^3$ with de-ionised water. are H_3BO_4 (1.4g); $MsSO_4.H_2O$ (1.8g); $ZnSO_4$ (0.22g); $CuSO_4.H_2O$ (0.08g); $NH_46MO_7.4H_2O$ (0.05g) and 0.01g of $FeCl_2$ (Alofe, 1985). For pH requirement, the basal medium was dispensed into $150cm^3$ milk bottles ($30cm^3$ per bottle) and pH adjusted to pH values 4.0-9.0 and autoclaved at $1.02kgcm^{-2}$ pressure at 121°C for 15 minutes. After cooling, each medium was inoculated with 0.7cm diameter mycelium from the actively growing culture of *S. commune* (5 day old) and incubated at 30 ± 2°C for seven days. Each treatment had three replicated.

For temperature requirement, the same basal medium was prepared and pH was regulated to 6.5 (found to be the best pH in the last experiment). The liquid medium (was dispensed) into milk bottles ($30cm^3$ per bottle) and autoclaved. After sterilization and cooling, each bottle was inoculated as described in pH experiment and incubated at 10, 15, 20, 25, 30, 35 and 45°C respectively. Each treatment was replicated three times after which the mycelia were harvested, oven dried at 55°C and weighed.

Carbohydrates

The ability of *S. commune* to utilise different carbohydrates as carbon sources for growth was also investigated by mycelia dry weight. The basal liquid medium employed was the same used for temperature and pH requirement studies. The basal medium was adjusted to pH 6.5 before being dispensed into $150cm^3$ milk bottles at the rate of $30cm^3$ per bottle. The bottles were sealed with aluminium foil and autoclaves at $1.02kg/cm^2$ and 121°C for 15 minutes. The carbon sources used included arabinose, fructose, galactose, glucose, mannose, sorbose, rhamnose, cellobiose, lactose, maltose, raffinose, sucrose cellulose, dextrin, starch, mannitol and myo-inositol. The basal medium without any carbon source served as the control. The appropriate weight of each carbon source to yield 1% carbon was autoclaved separately before adding aseptically to the sterile basal medium in the bottles.

Nitrogen Compounds

The basal medium used consisted of galactose (10.0g); KH_2PO_4 (0.5); $MgSO_4.7H_2O$ (0.5g); pyridoxine (500µg) and $1000cm^3$ of de-ionised water. Each nitrogen was supplemented at a concentration of nitrogen equivalent to that in 2.0g of sodium nitrate. For complex organic nitrogen sources (casein hydrolysate, peptone, urea, malt and yeast extracts), each was added at the rate of $2g/100cm^3$. The liquid medium without any nitrogen source served as the control.

Carbon/Nitrogen Ratio

The basal medium wassimilar to that used for testing nitrogen compounds except that galactose was omitted. Different ratios of galactose and casein hydrolysate (found to be the best in the previous experiment) were used. A concentration of 0.10g/1000cm^3 of galactose and casein hydrolysate in the basal liquid medium served as ratio 1:1. Other ratios were also prepared proportionately.

Analysis of Data

The data obtained were subjected to Analysis of Variance (ANOVA) and tests of significance was determined by Duncans Multiple Range Test.

Results and Discussion

Table 1 shows that *Schizophyllum commune* could grow within pH range of 4.0 and 8.5. Although, there were moderate growth between pH 5.5 and 7.5, optimum growth was recorded with pH 6.5. This result agrees with that of Plunkett,1953 and Aschan, 1954 who separately found that *Collybia velutipes* (an edible fungus) grew best within a narrow range of pH. Likewise, Fasidi (1996), obtained best mycelial growth of *Volvariella esculenta* with pH 6.0.. The optimum temperature obtained for mycelia growth of *S. commune* was 25°C although, this fungus was able to tolerate temperature of 10 – 40°C (Table 2). Chandra and Purkayastha (1977), reported 30 – 35°C as the optimum range for *V. volvaceae* mycelial growth with 32°C as the most suitable temperature. Fasidi(1996), also recorded 35°C as the optimum temperature for the growth of *V. esculenta*. Temperature and pH are important environmental factors that control the growth of fungi (Griffin, 1994). This ability to tolerate different pH and temperature probably enables *S. commune* to grow on various agricultural wastes at different geographical location of the world.

This fungus utilised various carbohydrate sources for its growth (Table 2). Galactose was the most stimulatory carbon source followed in order by mannitol and glucose ($P<0.05$). The utilization of galactose by *S. commune* was not a surprise because, this hexose sugar is an isomer of glucose which is commonly utilised by most fungi. Galactose could be easily isomerised to glucose during metabolism (Morrison and Boyd, 1992). Mannitol (the second best carbon source), has been implicated as the most suitable sugar alcohol that supports the growth of most fungi (Chandra and Purkayastha, 1977; Guha and Banerjee, 1971). Glucose (another stimulatory carbon source), has been widely reported as the most readily utilized carbohydrate for the growth of many mushrooms (Hong, 1978; Alofe, 1985; Jonathan and Fasidi, 2001b). Although, Kadiri and Fasidi (1994), reported dextrin as one of the most stimulatory carbon compounds for the growth of *Lentinus subnudus*, in this study, dextrin was least utilised. Its poor effect on the vegetative growth of *S. commune* could be attributed to the inability of this fungus to synthesise hydrolytic enzymes which can metabolise this complex carbon compound.

All the twenty-one nitrogen sources, used in this study were found to enhance mycelial growth of *S. commune* (Table 4). The most utilizable nitrogen compound was casein hydrolysate followed in order by peptone, ammonium nitrate and yeast extract ($P<0.05$). This result is similar to that obtained by Chandra and Purkayastha (1977), and that of Madunagu (1988) for *V. volvaceae* and *Pleurotus squarrosulus* respectively. The stimulatory effect of casein hydrolysate may be due to its amino acids composition (Bolton and Blair, 1982). It has been suggested that combined amino acids stimulated better growth than when applied singly (Nolan, 1970). peptone promoted second best vegetative growth among the studied nitrogen sources investigated (table 2). This complex organic source has been found to support the growth of many fungi (hong, 1978; Kadiri and Fasidi, 1994; Jonathan and Fasidi, 2001b). Of all the inorganicnitrogen sources tested, ammonium nitrate supported very good growth (136.7mg/30cm^3) while little growth wassustained by other inorganic nitrogen sources. On the other hand, most organic nitrogen sources enhanced very good growth (Table 4). This result implies that *S. commune* has a greater preference for organic than inorganic nitrogen compounds.

Among the tested carbon/nitrogen ratios, the best vegetative growth was obtained with ratio 5:2 (table 5). This result was different from that reported by Chandra and Purkayastha, 1977 (for *Agancus campestris*); Fasidi, 1996 (for *V. esculents)* and Jonathan and Fasidi, 2000a and b (For *P. atroumbonata* and *L. Procera*). This suggests that C:N ratios required for the growth of each mushroom may differ. The

result (Table 5) also revealed that *S. commune* utilized agricultural substrates that contained carbon/nitrogen at the ratios that could enhanced its growth. It could therefore be suggested that this fungus has its specific growth requirement.

In conclusion, the vegetative growth of *S. commune* was greatly improved by carbon and organic nitrogen sources at pH 6.5 and temperature of 25°C. Galactose and casein hydrolysate could be added into a growing medium in a ratios of 5:2. Agricultural substrates having ratio 5:2 can also be composted, this will enhance sporophore yield of *S. commune*.

Table 1: Effect of pH on Vegetative Growth of *S. Commune*

pH	Mycelial Dry Weight mg per $30cm^3$	Final pH of Medium
4.0	11.0 ± 2.8	4.9
5.0	31.7 ± 1.7	5.3
5.5	92.3 ± 3.3	5.3
6.0	126.7 ± 3.5	5.7
6.5	133.3 ± 2.3	5.8
7.0	96.7 ± 0.9	6.0
7.5	50.0± 1.3	6.7
8.0	23.3 ± 1.5	6.2
8.5	23.3 ± 1.5	6.6
9.0	10.0 ± 0.7	6.8

Values represented above are means of 3 replicates ± S.E at 1% level of probability.

Table 2: Effect of Temperature on vegetative Growth of *S. commune*

pH	Mycelial Dry Weight mg per $30cm^3$	Final pH of Medium
10	15.3 ± 3.3	6.7
15	35.0 ± 2.5	6.5
20	66.7 ± 3.8	5.9
25	150.0 ± 0.7	6.3
30	103.3 ± 1.2	5.7
35	43.3 ± 2.2	5.9
40	10.0 ± 1.6	6.1
45	-	6.2

Values represented above are means of 3 replicates ± S.E. at 1% level of probability.

Table 3: Effect of different carbon compounds on vegetative growth of *S. commune*

Carbon Compounds	Mycelial Dry Weight mg per $30cm^3$	Final pH of Medium
Monassacharides		
Arabinose	70.0 ± 5.8	6.9
Fructose	160.0 ± 5.8	6.3
Galactose	191.7 ± 4.4	5.8
Glucose	168.3 ± 3.3	6.4
Mannose	143.3 ± 3.3	7.3
Sorbose	100.0 ± 2.9	6.6
Rhamnose	80.0 ± 5.8	7.2
Oligosaccharides		
Cellobiose	100.0 ± 11.6	7.6
Lactose	70.0 ± 5.3	5.9
Maltose	81.7 ± 4.4	6.3
Raffinose	56.7 ± 2.9	5.8
Sucrose	60.0 ± 5.8	6.2
Polysaccharides		
Celluose	90.0 ± 0	6.8
Dextrin	60.0 ± 2.9	6.6
Starch	40.0 ± 2.7	7.5
Sugar Alcohol		
Mannitol	180.0 ± 5.0	6.7
Myo-inositol	88.3 ± 3.3	7.3
Control	30 ± 1.7	7.0

Values represented above are means of 3 replicates ± S.E. at 1% level of probability.

Table 4: Effect of various nitrogen sources on vegetative growth of *S. commune*

Nitrogen Compounds	Mycelial Dry Weight mg per $30cm^3$	Final pH of Medium
Amino Acids		
Aspartic acid	60.0 ± 2.2	6.3
Asparagine	53.3 ± 3.3	6.2
Alanine	60.0 ± 3.8	6.4
Glutamic acid	93.3 ± 3.8	6.0
Glutamine	100 ± 2.8	6.3
Cysteine	65.0 ± 5.8	6.7
Methionine	73.3 ± 2.9	6.4
Tryptophan	86.7 ± 4.7	6.5
Phenyl alanine	80.8 ± 5.3	6.3
Leucine	76.7 ± 3.3	6.0
Lysine	66.7 ± 2.9	6.4
Inorganic Compounds		
Ammonium nitrate	136.7 ± 4.8	5.7
Ammonium sulphate	76.7 ± 4.4	5.5
Calcium nitrate	60.0 ± 0.9	5.9
Potassium nitrate	60.0 ± 0.9	5.9
Sodium nitrate	50.0 ± 1.7	5.7
Complex Organic Compounds		
Casein hydrolysate	185.0 ± 2.7	6.6
Malt extract	93.3 ± 3.3	6.7
Peptone	150.0 ± 5.8	6.8
Urea	66.7 ± 4.8	6.5
Yeast Extract	110.0 ± 5.8	7.0
Control	46.7 ± 3.3	6.7

Values represented above are means of 3 replicates ± S>E. at 1% level of probability.

Table 5: Effect of different carbon/nitrogen ratios on vegetative growth of *S. commune*.

Carbon/Nitrogen Ratio	Mycelial Dry Weight mg per $30cm^3$	Final pH of Medium
1:2	60.0 ± 2.9	6.4
1:2	56.7 ± 3.3	6.3
1:3	53.3 ± 3.3	6.1
1:4	56.7 ± 4.7	6.7
1:5	50.0 ± 0	5.6
2:1	100 ± 2.2	5.8
2:3	80.0 ± 5.8	6.0
2:5	73.3 ± 3.3	6.3
3:1	100.0 ± 0	6.4
3:2	120.0 ± 11.6	6.7
3:4	96.7 ± 3.3	6.2
3:5	90.0 ± 1.4	6.4
4:1	100.0 ± 2.2	6.8
4:3	160.0 ± 5.8	6.9
4:5	146.7 ± 3.3	6.3
5:1	140.0 ± 8.8	6.1
5:2	203.3 ± 8.1	6.2
5:3	150.0 ± 0.9	6.3
5:4	116.7 ± 3.3	6.6
0:0 (Control)	45 ± 4.1	7.0

Values represented above are means of 3 replicates ± S.E. at 1% level of probability.

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