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OPTIMIZATION OF MYCELIAL GROWTH OF *PLEUROTUS SAJOR-CAJU* (Fr.) SINGER IN RELATION TO SOME PHYSICAL AND NUTRITIONAL FACTORS

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Dry weight yield of mycelium of *Pleurotus sajor-caju* (Fr.) Singer grown in submerged liquid culture was found to be greatest under the following conditions: 25°C temperature, complete darkness, phosphate buffer at pH 5.5, starch or dextrin as carbon source, asparagine as nitrogen source. Glucose and sucrose were poor carbon sources for this fungus: inorganic nitrogen sources gave satisfactory growth.

Key words : Mycelial Growth, Pleurotus sajor-caju, Physical and Nutritional factors

INTRODUCTION

Mushroom cultivation is gradually becoming popular from a socio-economic point of view in developing countries like India, and significant progress has been made in mushroom research during the last two decades (Bano and Srivastava, 1962; Jandaik and Kapoor, 1974; Bano *et al*, 1979; Sivaprakasam 1986; Samajpati *et al.*, 1986) ; but certain aspects of mushroom research particularly concerning the production of mycelial biomass still receive little attention. Both intensive and extensive researches are imperative to determine the factors stimulating and accelerating the growth of edible mushroom mycelia in liquid culture for propagation and biochemical investigations. The purpose of the present study is to (a) evaluate the effect of different factors on mycelial growth of *P. sajor-caju* in liquid culture, (b) establish optimal conditions for mycelial growth of *P. sajor-caju*.

MATERIALS AND METHODS

Test Organism

A tissue culture of *Plrurotus sajor-caju* (Fr.) Singer was prepared from the fresh sporocarp of a mushroom obtained from Santoshpur, West Bengal during the rainy season. The cultures were isolated into 3% malt agar medium and maintained on

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the same medium by subculturing regularly at 30 day intervals. The cultures were kept at 25 C in complete darkness. Glucose-asparagine medium (Lilly and Barnett, 1951) was used as basal liquid synthetic medium without salts of iron, zinc and manganese.

Preparation of inoculum

A small portion of actively growing mycelium from agar slants was aseptically transferred to a 250 ml Erlenmeyer flask containing 50 ml of sterile basal liquid synthetic medium, and then incubated on a shaking incubator (120 rpm) at 30 C (± 1 C) for 7 days in complete darkness (Bhattacharjee and Samajpati, 1988). After the incubation period, the mycelial mat was aseptically fragmented into small pieces with the help of a waring blender. Fragmented mycelial mass was then washed several times with distilled water to remove any trace of medium and suspended in a phosphate buffer medium (pH 5.5) for 24 h to overcome the shock encountered during blending. One ml of this mycelial cell suspension was then used as inoculum.

Experimental Growth Conditions

50 ml aliquots of the liquid basal medium were placed into 250 ml of Erlenmeyer flasks, the pH of the medium adjusted to 5.5 by 0.2 M phosphate buffers and the flasks plugged and sterilized at 10 lbs p.s.i. for 20 minutes. Each flask was then inoculated with 1 ml mycelial cell suspension of the test fungus and incubated (with shaking at 120 rpm) in complete darkness for 15 days. Several flasks were inoculated in order to give five replicates of each treatment.

Measurement of Growth

After 15 days of incubation, the five flasks of each treatment were harvested. The medium and the mycelium was separated by filtration through a tared sintered funnel (Jena IG 3). The filtered mycelium was washed repeatedly with distilled water to make it free from any trace of adherent medium, then dried at 60 C for 96h in an oven, cooled in a desiccator and weighed. Dry weight of mycelium thus obtained was taken as an index af growth.

RESULTS

Physical factors

Effect of temperature

The flasks were inoculated with 1 ml of mycelial cell suspension and incubated at different temperatures for 15 days. Dry weights of the mycelium were recorded and presented in Table 1.

The results reveal that 25°C is the optimum temperature for the mycelial growth. Mycelial growth gradually decreased above 25°C and was completely checked at 40°C and onwards.

Table 1. *Effect of temperature on the mycelial growth (g/l) of* P. sajor-caju *after 15 days incubation*

Temperature (°C)	Dry weight of the mycelium (g/l)* with S.E.
15	0.430±0.036
20	4.416±0.046
25	7.860±0.042
30	6.030±0.040
35	0.540±0.032
40	Nil
45	Nil

*Data are average of five replicates/treatment

Effect of light

250 ml conical flasks, each containig 50 ml of sterile glucose asparagine liquid medium were inoculated with 1 ml of mycelial cell suspension and incubated at 25°C under different illuminations.

Average dry mycelial weights of 5 replicates, 15 days after harvest are presented in Table 2. The results reveal that maximum mycelial growth occurred under continuous darkness while minimum growth occurred under continuous strong light.

Table 2. *Effect of light on the mycelial growth* (g/l) *of* P. sajor-caju *after 15 days incubation*

Light Sources	Dry weight of the mycelium (g/l)* with S.E.
continuous light (225-275 lux)	5.480±0.030
continuous dark (0 lux)	6.950±0.050
continuous diffused light (125-150 lux)	6.260±0.050
Alternate 12 hrs light and 12 hrs darkness (225-275 lux and 0 lux alternately)	6.550±0.042
Natural normal room light and darkness (75-100 lux and 0 lux alternately)	5 840±0.050

*Data are averege of five replicates/treatment

Effect of pH

250 ml conical flasks each with 50 ml of basal medium were adjusted to different pH by buffering with 0.2 M phosphate buffer. Each of these flasks (sterilized) were then inoculated with 1 ml of mycelial cell suspension and incubated for 15 days at 25°C under complete darkness. The results presented in Table 3 shows that *P. sajor-caju* prefers acidic conditions and showed optimum growth at pH 5.5.

Nutritional factors

Effect of carbon sources

The basal medium (without carbon) was supplemented separately with different carbon sources. In each case an amount of carbon equivalent to that present in

2.5% glucose (25 g/l) was used in the medium. The basal medium without carbon served as cantrol. The results presented in Table 3 show that starch and dextrin gave the maximal mycelial growth. D-xylose also gave very good growth. Growth on glucose was less than half that of growth on the above.

Table 3. *Effect of pH on the mycelial growth (g/l) of* P. sajor-caju *after 15 days incubation*

pH	Dry weight of the mycelium (g/l)* with S E.
4.0	2.900±0.036
4.5	3.360±0.044
5.0	3.950±0.050
5.5	4.780±0.060
6.0	4.210±0 075
6.5	3.700±0.045
7.0	2.610±0.060
7.5	1.210±0.025

*Data are average of five replicates/treatment

Table 4. *Effect of different carbon sources on the mycelial growth (g/l) of* P. sajor-caju *after 15 days incubation*

Carbon Source	Dry weight of the mycelium (g/1)* with S.E.
Glucose	4.120±0.038
Lactose	5.000±0.026
Maltose	6.800±0.075
Fructose	4.000±0.040
Surcrose	2.910±0.046
D-galactose	2.640±0.040
D-xylose	9.700±0.060
Dextrin	10.200±0.0:0
Starch	10 280±0.080
Carboxymethyl cellulose	7.680±0.050
D-ribose	5.560±0.065
Control (without carbon source)	0.360±0 020

*Data ate average of five replicetes/treatment

Effect of nitrogen sources

Different nitrogen compounds both organic and inorganic were separately added to the basal medium at a concentration of nitrogen equivalent to that in 2 g asparagine. The results in Table 5 show that *P. sajor-caju* preferred L-asparagine for optimal growth, but that all nitrogen sources gave rather similar yields of mycelium.

Table 5. *Effect of different nitrogen sources on the mycelial growth (g/l) of* P. sajor-caju *after 15 days incubation*

Source of nitrogen	Dry weight of the mycelium (g/1)* with S.E.
NH_4NO_3	8.875.±0.100
KNO_3	9.100±0.020
$(NH_4)_2SO_4$	8.865±0.025
$Ca(NO)_3)_2$	8.850±0.020
$NaNO_3$	8.895±0.025
Asparagine	10.600±0.100
Peptone	9.500±0.030
Urea	8.750±0.040
Casein hydrolysate	9.200±0.020
Yeast extract	9.150±0.015
Control (without nitrogen)	3.200±0.030

*Data are average of five replicates/treatment

DISCUSSION

Physical factors have significant influence on the growth of mushroom mycelia in liquid culture. The present investigation revealed that the best mycelial growth was obtained at an optimum temperature of 25°C. Kaufert (1935, 1936) reported that in *P. corticatus* the mycelium grew about 5% as fast at 10°C as it did at the optimum 27°C, but that it grew at about 75% of the optimum rate at 32°C. There has been no recent report of attempts to cultivate the species ; however, the original identification has been doubted (Kurtzman and Zadrazil, 1978). The optimum temperature for mycelial growth of *P. cystidiosus* was approximately 27°C (Jong and Peng, 1975). In case of *P. ostreatus* the optimum growth for mycelium was at 20-30°C (Zadrazil, 1976). Although *P. tuber-regium* has been cultivated only on a primitive basis, the mycelial growth has been found to be optimal at 35°C, with no growth at 10°C or 45°C (Oso, 1977).

Continuous darkness favours the highest yield of mycelia as compared to the continuous light condition which has an inhibitory effect on the mycelial growth. *P. sajor-caja* is an acid loving fungus showing optimum mycelial growth at pH 5.5 and show gradually reduced growth towards the alkaline side. Sugimori *et al.* (1971) found that an initial pH of 4 to 5 was optimum for *P. ostreatus*. The optimum initial pH for *P. eryngii* was 5.0 while the optimnm initial pH for *P. florida* was 6.0 (Zadrazil, 1978). For *P. flabellatus* Srivastava ahd Bano (1970) found an optimum initial pH of 4.5 to 6.5.

Like many other edible mushrooms this fungus also prefers polysaccharide rather than monosaccharide. Starch was found to be the best carbon source followed closely by dextrin and D-xylose. Jandaik and Kapoor (1976) reported that highest growth was supported by starch, but good growth was also supported by maltose, glucose, sucrose and dextrin but their basal medium contained 3% of each carbohydrate and completely free of organic compounds. Reports of other *Pleurotus* species show rather less agreement. In *P. ostreatus* greatest growth was supported by mannose followed by starch, glucose and maltose (Hashimoto and Takahashi 1976). In *P. stipticus* and *P. ostreatus* all monosaccharides supported growth, but arabinose and xylose supported less than 40% of the growth of both species in glucose medium (Rypacek, 1977). In *P. ostreatus* glucose, maltose and mannitol supported good growth at 10% sugar content in the basal medium (Hong, 1978). In the case of *P. flabellatus* highest growth was supported by mannose followed by fructose and glucose at 1.5% sugar content in the basal medium (Oso, 1977). In *P. tuber-regium* maximum growth was supported by fructose followed by mannose and cellobiose at 2% suger content in the basal medium. The difference, as reported by various authors in the ability of *Pleurotus* cultures to use carbon sources may be an expression of the physiological differences in the species, or of the isolates of the same species might give different results (Kurtzman and Zadrazil, 1978).

Nitrogen is very important to the growth of all organisms and in fungi it is also necessary for the chitin in the cell walls (Kurtzman and Zadrazil, 1978). In the present investigation on *P. sajor-caju* asparagine was found to be the best nitrogen source among the tested compounds. This confirms the earlier report of Jandaik and Kapoor 1976) who reported that at 0.03% nitrogen concentration in the basal medium asparagine supported the maximum yield. Urea was found to support the greatest growth in experiments with nitrate and ammonium salts, as well as a number of organic sources of nitrogen (Eger, 1970 ; Jandaik and Kapoor, 1976 ; Voltz, 1972). Dibasic ammonium phosphate was found to support more growth of *P. ostreatus* than any other pure compound studied by Hong (1978). Ammonium citrate supported more growth of *P. ostreatus* than any other salt tested by Voltz (1972). Ammonium tartrate supported more growth of *P. ostreatus* than any other salt tested by Hashimoto and Takahashi (1976). The data of various authors who studied amino acids as the sources of nitrogen are far more inconclusive, when they are compared, than the data on salts (Kurtzman and Zadrazil, 1978). Asparagine and serine (Hashimoto and Takahashi, 1976 ; Hong, 1978 ; Jandaik and Kapoor, 1976e), aspartic acid (Hong, 1978 ; Voltz, 1972), and alanine (Eger, 1970 ; Hashimoto and Takahashi, 1976 ; Hong, 1978 ; Sugimori *et al* , 1971 ; Voltz, 1972) promoted more growth than most of the other amino acids.

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