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Growth of mycelial biomass and fruit body cultivation of *Lentinus squarrosulus* collected from home garden of Tripura in Northeast India

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ABSTRACT

The present study aimed at cultivating the fruiting body of an edible mushroom identified as *Lentinus squarrosulus* collected from home garden of Tripura in Northeast India and for assessing the mycelium biomass growth using various carbon and nitrogen sources under submerged culture condition. The cultivation of the mushroom using paddy straw revealed dry weight of 0.25±0.02 g per fruit body with 21.83 % of biological efficiency. Under submerged culture condition, fructose exhibited highest mycelium dry weight and yeast extract was found to be a best nitrogen source. The availability of the mushroom in the forest and homegarden of Tripura may be utilized for its commercial cultivation and inclusion in the local food. Fructose and yeast extract is recommended for high mycelial biomass production.

1. INTRODUCTION

Wild mushrooms are gaining importance in modern diet for their nutritional and pharmacological properties [1]. Many species of *Lentinus* are reported to be edible which can be cultivated on pasteurized as well as unpasteurized substrates [2]. Moreover, species of *Lentinus* have been reported to grow on various substrates [2-6].

The wild edible mushroom was collected from the homegarden of Tripura, Northeast India. A home garden refers to the traditional land use system around a homestead where several species of plants are grown and maintained by the household members and their products are primarily intended for the family consumption [7]. Homegarden with trees are one of agroforestry practices known to be ecologically sustainable and diversifies livelihood of local community.

In the present work, a naturally occurring strain of *Lentinus squarrosulus* (Mont.) Singer was collected from the wild and an attempt was made to cultivate the fruit body. In addition, the effect of different carbon and nitrogen sources on its mycelium growth and biomass production under submerged culture condition was assessed.

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2.MATERIALS AND METHODS

2.1 Sample collection

An edible mushroom was collected from the homegarden of Tripura (latitude 23°47'48.51"; longitude 91°16'20.40"; 24 m.a.s.l) in Northeast India during April-October, 2013. Collected samples were packed in sterilized polythene zipped bag and brought to the laboratory for their identification. Identification of the specimens was carried out by considering various morphological characteristics [8] and molecular marker.

The mushroom was identified as *Lentinus squarrosulus* using the molecular marker sequences and the sequences were deposited in NCBI Gene Bank (Accession Number KP340800). Dried specimens were preserved as herbarium material (MCCT 03) in the Mycology and Plant Pathology Laboratory of the parent University.

2.2Tissue culture

The material was washed thoroughly with autoclaved deionized water and 80% ethanol. It was then tap dried with sterile blotting paper. The middle portion between stipe and pileus of the mushroom was cut under sterile condition in the laminar air flow and inoculated in the malt extract agar slants and incubated at 25°C for two weeks.

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2.3 Growth of mycelial biomass under submerged culture conditions with various carbon and nitrogen sources

Lentinus squarrosulus were maintained on malt extract agar at 25°C. All submerged culture was conducted in 100 ml Erlenmeyer flasks filled with 30 ml of basal synthetic medium. Fungal inocula were grown on synthetic medium consisting of the following components (g/l of deionized water): glucose-30.0, yeast extract-2, peptone-2.5, $MgSO_4.7H_2O$-0.5, $Ca(NO_3)_2$-0.5, $(NH_4)_2SO_2$-0.25, $FeCl_3$-0.010, $ZnSO_4$-0.005, inositol-0.050, thiamine-0.0001, biotin-0.0005, folic acid-0.0001 and $CaCl_2$-2.944. To study the effect of carbon sources on the fungal growth; in the basal synthetic medium, various carbon sources such as Carboxy methyl cellulose, Dextrose, Fructose, Lactose, Maltose, Starch, Sucrose and Xylose were tested. To study the effect of nitrogen sources, various amino acids and complex nitrogen sources such as Ammonium nitrate, Casein, Peptone, Urea, Yeast Extract, Alanine, Arginine, Asparagine, Glycine, Methionine, Tryptophan and Tyrosine were evaluated. Controls without carbon or nitrogen sources, respectively, were run in parallel. The flasks were inoculated with two week old precultures (0.5 cm^3) and submerged cultures were kept stationary at 28°C for 14 days. The dry weight and growth rate was calculated.

2.4 Cultivation

For the production of spawn, 200 g of wheat grains was boiled with 500 ml of water for 20 mins. The water was then drained off the grains and was surface dried for few hours. After drying the grains were thoroughly mixed with 2% calcium sulphate and 0.5% calcium carbonate. The mixture was filled in the 500 ml conical flasks and plugged for autoclaving. The mycelium was inoculated in the wheat grain for the production of spawn in darkness for 3 weeks. At regular period of time the conical flasks were shaken for uniform growth of mycelium. The cultivation of fruit body of *L. squarrosulus* was initiated by soaking the paddy straw overnight. The water drained paddy straw was chopped (10 cm in length) and was autoclaved next day. The paddy straw was placed in 10 kg polythene bag in layers. The first layer of paddy straw was followed with the placement of wheat spawn alternately. The tied bag was incubated for three weeks at 32(±2)°C. The fruit bodies were harvested and the length and dry weight were determined after incubation period along with its biological efficiency [6].

3. RESULTS AND DISCUSSION

The source for the spawn production and inoculum for mycelium growth was obtained from the fruit body found growing in the dead mango tree in a home garden (Fig. 1a). The flushing of the fruit body after cultivation using paddy straw was observed after three weeks (Fig. 1b&c). The stipe length, pileus diameter, dry weight and biological efficiency is presented in Table 1. The emergence of juvenile fruit bodies in malt extract media in the test tube was found after 60 days (Fig. 1d). Upadhyay and Rai (1999) reported wheat straw and paddy straw compost for the cultivation of *L. squarrosulus*. Oghenekaro et al. (2009) documented successful cultivation of *L. squarrosulus* on sawdust of five economic tropical tree species. The characterized mushroom was capable of utilizing all the tested carbon and nitrogen sources but the mycelial growth was greatly affected by the compound used in the nutrition medium. Carbon sources are major components of the nutrient medium, which must ensure the best growth of fungi. Submerged culture has a number of advantages including higher mycelial yield in a more compact space and shorter time, with fewer chances of contamination [9-11] which is now attracting attention as an alternative for efficient yield of mycelia and polysaccharide [12]. The mycelial growth of edible mushroom using different carbon and nitrogen source under submerged culture condition was tested using basal synthetic media. In case of carbon, maximum growth was observed using fructose as a source and minimum was carboxy methyl cellulose (Table 2).

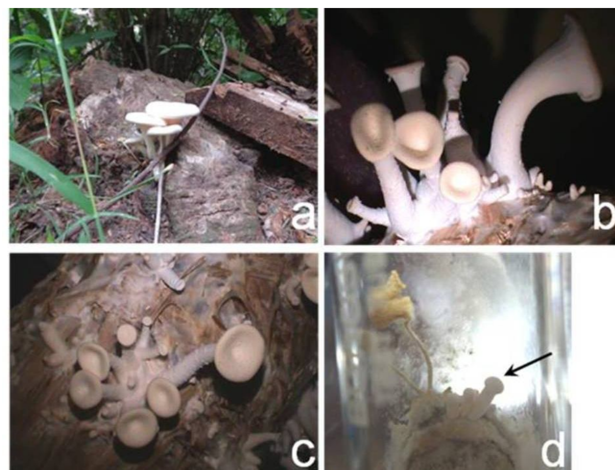

Fig. 1: (a) Mushroom fruit body growing in home garden. (b & c) Fruit body arising from the paddy straw substrate under cultivation. (d) Fruit body developing from mycelium in malt extract media in test tube.)

Table 1. Fruit body features of an edible mushroom cultivated using paddy straw after first flushing.

Fresh weight (g)	Dry weight (g)	Stipe length (cm)	Stipe diameter (cm)	Pileus diameter (cm)	No. of fruit body	Biological efficiency (%)
1.14±0.10	0.25±0.02	2.55±0.13	0.33±0.02	3.15±0.15	15	21.83

Table 2: Mycelial growth of edible mushroom using different carbon source under submerged culture condition.

Carbon Source	Dry weight (g/l)	Growth rate (g/l/day)
Control	1.67±0.27	0.12
Carboxy methyl cellulose	7.33±0.53	0.52
Dextrose	14.95±0.75	1.07
Fructose	17.45±0.02	1.25
Lactose	15.63±0.57	1.12
Maltose	17.12±1.55	1.22
Starch	15.90±1.33	1.14
Sucrose	16.62±0.75	1.19
Xylose	12.70±0.20	0.91

The dry weight ranged between 4.20 and 17.45 g/l using different carbon sources (Fig. 2). In case of nitrogen sources, maximum dry weight was observed in yeast extract and minimum was in ammonium nitrate (Table 3) and it ranged from 0.20 and

9.77 g/l (Fig. 3). As reported earlier [13], fructose and yeast extract were proved to be more suitable than other carbon and nitrogen sources which is in agreement with the present study.

Table 3: Mycelial growth of edible mushroom using different nitrogen source under submerged culture condition.

Nitrogen Source	Dry weight (g/l)	Growth rate (g/l/day)
Control	0.34±0.04	0.02
Ammonium nitrate	0.20±0.03	0.01
Casein	5.37±1.30	0.38
Peptone	5.68±1.45	0.41
Urea	1.02±0.15	0.07
Yeast Extract	9.77±0.10	0.70
Alanine	0.50±0.30	0.04
Arginine	0.90±0.63	0.06
Asparagine	0.23±0.10	0.02
Glycine	0.30±0.00	0.02
Methionine	0.39±0.02	0.03
Tryptophan	0.45±0.15	0.03
Tyrosine	0.55±0.05	0.04

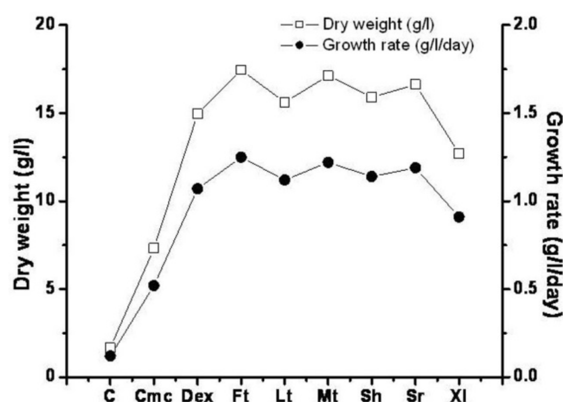

Fig. 2: Dry weight of mycelium using different carbon sources under submerged culture condition (C-Control, Cmc-Carboxy methyl cellulose, Dex-Dextrose, Ft-Fructose, Lt-Lactose, Mt-Maltose, Sh-Starch, Sr-Sucrose, Xl-Xylose.

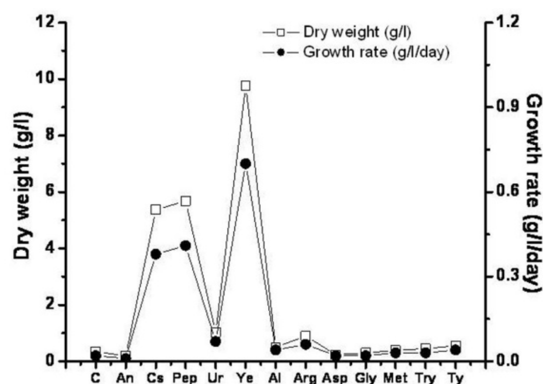

Fig. 3: Fig. 3: Dry weight of mycelium using different nitrogen sources under submerged culture condition (C-Control, An-Ammonium nitrate, Cs-Casein, Pep-Peptone, Ur-Urea, Ye-Yeast Extract, Al-Alanine, Arg-Arginine, Asp-Asparagine, Gly-Glycine, Met-Methionine, Try-Tryptophan, Ty-Tyrosin.

4. CONCLUSION

The results of the present study on the local mushroom supplements the fact that *L. squarrosulus* can be successfully cultivated in the laboratory on lignocellulosic substrates such as paddy straw. This can be widely exploited for industrial use in the production of mycelium with fructose and yeast extract as carbon and nitrogen sources, respectively.

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