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STUDY OF DIFFERENT GROWTH PARAMETERS IN *GANODERMA LUCIDUM*

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ABSTRACT

***Ganoderma lucidum* was grown in several culture media, using four different concentrations of inoculum. The mycelia grew well in the potato dextrose medium at pH 5 and 25 C, followed by malt extract, Kirk medium + molasses, and Kirk medium + glucose. The yield of mycelial biomass (dry weight basis) in potato dextrose medium after 15 days was about 1.59 g/100 ml; in malt extract, 0.91 g/100 ml; in Kirk molasses, 0.68 g/100 ml; and in Kirk glucose was 0.38 g/100 ml. Mycelial growth of *G. lucidum* was significantly higher in case of 5 to 15 ml inoculum concentration.**

Key words: *Ganoderma lucidum*, potato dextrose, malt, molasses, Kirk medium.

INTRODUCTION

Ganoderma lucidum has been known in Japan, China and other countries as a food and raw material for the development of drugs[9]. It is a shelf-like mushroom that grows on dead and dying trees. The fruit body of *G. lucidum* is employed medicinally. It occurs in six different colors, but red variety is most widely used and commercially cultivated in North America, China, Taiwan, Japan, and Korea.

The global production of *G. lucidum* was estimated to be 4900–5000 tons in 2002, and at least 100 brands of *G. lucidum* products are sold on the market[9]. In addition, attempts are being made to obtain useful mycelial products or to produce effective substances into a medium by means of liquid culture of the mycelia. Mycelial biomass powder can be used to formulate various types of health tablets and capsules[2]. Many medicinal and edible mushrooms are capable of growing in the form of mycelial biomass in submerged culture[10]. The mycelial biomass obtained by submerged cultivation of many species also has higher nutritional value. Mushroom mycelium has been grown in wastes from the processing

Table 1. Media composition.

Constituents	Potato dextrose (PD)	Malt extract (ME)	Kirk[8] + molasses (KMM)	Kirk[8] + glucose (KMG)
KH_2PO_4			2.1 g/l	2.1 g/l
$MgSO_4$			0.3 g/l	0.3 g/l
$Ca\ Cl_2\ .2H_2O$			0.4 g/l	0.4 g/l
$(NH_4)_2\ SO_4$			2.0 g/l	2.0 g/l
Yeast extract		4.0 g/l	0.4 g/l	0.4 g/l
Glucose	10.0 g/l			20.0 g/l
Molasses			4.4 g/l	
Potato infusion	200.0 g/l			
Malt extract		10.0 g/l		

citrus fruits, in molasses, and in sulfite waste liquor from the paper and pulp industries[7]. Culture media, in which mycelium grows, may be made of chemically pure and ecologically clean substances. The cultivation of mushrooms for fruit body production is a long-term process, taking one to several months for the first fruit bodies to appear, depending on species and substrate. By contrast, the growth of pure mushroom cultures in submerged condition in liquid culture media permits acceleration of the growth, resulting in a biomass yield in several days[10].

In this paper we studied the mycelial growth of *G. lucidum* on different culture media, the consumption of molasses, and the effect of different concentration of inoculum in submerged cultivation.

MATERIALS AND METHODS

Strain of *Ganoderma lucidum* (Leysser)Karsten, 64489, used in this study was provided by the National Research Council of Canada, Prairie Regional Laboratory, Saskatchewan, Canada. It was maintained on potato dextrose agar medium in test tube slants at 8-10 C.

Composition of media. The composition of media used for the maintenance of the *G. lucidum* cultures and fermentation studies are given in **Table 1**. The pH of all media were adjusted to 5 with 1N HCl or NaOH. Double distilled water was used in all experiments. All the media were autoclaved at 121 C for 15 minutes.

Cultivation of organism. Aliquots of 100 ml of each prepared medium were transferred to 250-ml Erlenmeyer flasks. For homogeneous culture, 0.5 cm diameter biomass discs (6 in each flask) were inoculated using a sterilized cork borer. Discs were taken from actively growing cultures. In the second step 5, 10, 15 and 20-ml were inoculated to the flasks. Flasks were shaken mechanically on an Eyela NT-331 shaker at 100 rpm and 25 C to mix the flask contents and for aeration. Cultures were allowed to grow. The 100 ml fermentation broth was centrifuged at 3000 rpm and the supernatant liquid neutralized with 0.1M-HCl or NaOH for the estimation of residual molasses.

Estimation of total sugar. Sugar was estimated according to the method of AOAC[1].

Statistical analysis. Mean values of

Table 2. Growth of *Ganoderma lucidum* on different media.

Medium	Growth, dry weight, g/100ml				
	3 days	6 days	9 days	12 days	15 days
PD	0.19 b	0.42 a	0.50 a	0.65a	1.59 a
ME	0.26 a	0.35 b	0.42 b	0.58 b	0.91 b
KMM	0.15 c	0.22 c	0.38 c	0.55 c	0.68 c
KMG	0.08 d	0.09 d	0.15 d	0.28 d	0.38 d

Means with different letters in a column show significant difference (P=0.05) as determined by DMR Test.

parameters studied were analyzed by the Duncan Multiple Range Test (DMR)[6]. The data on consumption of molasses sugar were analyzed statistically by T test.

RESULTS AND DISCUSSION

The hyphal growth of *G. lucidum* on different culture media is shown in **Table 2**. Favorable conditions for vegetative growth were provided in shake flask culture. Maximum mycelial growth after 15 days (1.59 g) was recorded on potato dextrose medium (PD) at pH 5 and 25 C, which was significantly higher than in Kirk medium + molasses (KMM, 0.68 g), malt extract (ME, 0.91 g), and Kirk medium + glucose (KMG, 0.38 g).

The fungal mycelium grew in the form of spherical ball with spine, brown and yellowish brown in color. The maximum mycelial growth occurred sooner in PD than other media. The results indicate that PD was favorable to the greatest mycelial yield of the mushroom, followed by ME. In KMM and KMG fungal mycelial growth was weak.

Table 3 shows the effect of inoculum concentration on the growth of *G. lucidum.* The fungal growth significantly increased in concentration of 5, 10 and 15 ml of inoculum in PD, but decreased abruptly with 20 ml. Similar results were obtained by Reusser *et al.*[4,5]. Similarly in ME, KMM, and KMG the fungal biomass was greater with 5 and 10 ml inoculum concentration, but decreased in 15 and 20 ml concentration. Data showed clearly that 20 ml concentration in PD, while 15 and 20 ml in ME, KMM, and KMG, inhibit mycelial growth of *G. lucidum* in pure culture. It can be concluded that suitable concentrations of inoculum in the culture media should be provided in order to obtain optimal mycelial growth[3].

Table 4 shows the growth of *G. lucidum* on KMM, using molasses as a carbon source. The consumption of molasses sugar increased significantly after 3 to 15 days of incubation in comparison with the control. The dry weight of fungal biomass also increased from 4.7 g to 9.11 g during this period. Similar results were obtained in *Tricholoma nudum* cultures using simple nitrogen and sugar cane molasses as a carbon and energy source[5].

Hence it can be concluded from this study that PD was the best for the mycelial growth of *G. lucidum* using an inoculum concentration of 15 ml.

Table 3. Effect of inoculum concentration on the *Ganoderma lucidum* growth.

Medium	Inoculum Conc./100 ml	Growth, g/100 ml 3 days	6 days	9 days	12 days	15 days
PD	5 ml	0.12 d	0.20 d	0.36 d	0.61 d	1.36 d
	10 ml	0.18 c	0.28 c	0.55 c	0.88 c	1.84 b
	15 ml	0.51 a	1.18 a	1.68 a	1.79 a	2.37 a
	20 ml	0.25 b	0.68 b	0.75 b	1.19 b	1.47 c
ME	5 ml	0.13 b	0.26 b	0.43 a	0.49 b	1.01 b
	10 ml	0.14 a	0.32 a	0.45 d	0.52 a	1.03 a
	15 ml	0.13 b	0.24 b	0.28 b	0.43c	0.47 c
	20 ml	0.09 c	0.16 c	0.16 c	0.33d	0.43d
KMM	5 ml	0.09 c	0.10 c	0.12 c	0.30 c	0.57 b
	10 ml	0.11 b	0.18 a	0.37 b	0.51 a	0.75 a
	15 ml	0.14 a	0.15 b	0.16 b	0.44 b	0.40c
	20 ml	0.13a	0.14 b	0.14 b	0.21 d	0.22 d
KMG	5 ml	0.12 a	0.15 ab	0.17 b	0.26 b	0.42 a
	10 ml	0.11b	0.16 ab	0.22 a	0.31 a	0.46 a
	15 ml	0.12 a	0.13 a	0.04 c	0.19 c	0.2 b
	20 ml	0.03 c	0.11b	0.13 b	0.17 b	0.18 b

Means with different letters in a column show significant difference (P=0.05) as determined by DMR Test.

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Table 4. Estimation of molasses sugar consumed by *Ganoderma lucidum.*

Time period	g /l Control	3 days	6 days	9 days	12 days	15 days
Consumption of molasses	4.40	43.10	36.0	32.30	25.80	22.40
Dry weight of biomass	0.0	4.70	5.50	7.61	8.96	9.11