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# Effects of physical factors and synthetic media on mycelial growth of *Lyophyllum decastes*

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**Abstract**

We investigated the effects of light, moisture, amino acids, vitamins and mineral nutrients on mycelial growth of the medicinal mushroom *Lyophyllum decastes*. Mycelial growth experiments were carried out on solid media. The best mycelial extension was recorded in total dark conditions, followed by 12 h alternating light/dark and total light, in respective order. This fungus grew well on substrate containing 65% moisture. Of the eight amino acids tested, the best growth (61.73 mm, after two-week incubation) was supported by glutamic acid, followed by proline and alanine. Six vitamin sources were used and riboflavin was the most utilizable (56.57mm). $CaSO_4$ was found to be the most favorable mineral source for growth (67.80 mm).

## 1. Introduction

Fungi are currently of interest because they are biologically rich sources of various active substances [1]. Mushrooms have become attractive as additional functional food and as source for the development of drugs. Recently, *Ganoderma lucidum*, a medicinal mushroom, has appeared in Nepalese markets in capsule form. *Lyophyllum decastes* (Fr) Sing., a basidiomycete that belongs to the family *Tricholomataceae*, is a medicinally potent mushroom. Eleven polysaccharides were isolated from a hot water extract of *L. decastes* fruit body. Among them, three polysaccharides had marked antitumor activity against Sarcoma 180 [2].

The nutritional requirements for mushroom mycelium are relatively simple. In the growth of fungi, physical factors greatly affecting it are temperature, light, moisture, and aeration [3]. Similarly, various nutrient supplements play an essential role in metabolism for their functions as coenzymes. Therefore, mycelial growth is a preliminary step that creates suitable internal conditions for fruiting. Thus, outstanding growth of mycelium is a vital factor in mushroom cultivation.

Majority of studies on mycelial growth of mushrooms in recent times have been carried out using a submerged fermentation method which, however, might not reflect the mycelial growth condition occurring on solid state culture [4]. Solid state culture fermentation (SSF) has several advantages over liquid culture [5]. Technology for synthetic cultivation of *L. decastes* on livestock compost has been developed [6]. However, other techniques for its culture and its nutrient substrates are still being explored. In the present study, we investigated the suitability of physical factors (*e.g.*, light, moisture) and various nutrient supplements for mycelial growth of *L. decastes*.

## 2. Materials and Methods

The experimental organism used in this study was *Lyophyllum decastes* (Fr) Sing. (KS-74, originally obtained from Kyushu University, Japan [6].

### *2.1. Effect of light*

Effects of light on mycelial extension were determined by measuring the radial growth of the colony. Potato dextrose agar (PDA) medium was autoclaved at 121°C for 15 min, and 20 ml of it was poured into petri dishes. After cooling, a 5 mm diameter disk of actively-growing mycelia from PDA was transferred into the medium, and then incubated at 25°C for two weeks in three different light conditions in the incubator (fluorescent light). The light conditions were: a) first group was incubated in total darkness, b) the second group was in complete light, and c) the third group was in 12 h alternating

shifts of total darkness and light. Colony diameter was recorded after two weeks of incubation.

*2.2. Effect of moisture content and sterilization*

Effects of moisture on mycelial extension were tested on substrate based media. For that, dried fermented compost [6] and sawdust were mixed in a ratio of 1:1 (w/w), and a mixture of wheat, rice and barley bran (ratio of 1:1:1 w/w) was used as a supplement at 20%. To prepare a substrate containing 50% moisture, 50 ml of tap water was added to 100 g of the substrate and mixed thoroughly. In this way, substrates with 40%, 60%, 65%, 70%, and 80% moisture contents were prepared. Substrates (100 g) of different moisture contents were placed into the petri dishes and were autoclaved at 121 C for 90 min. The plates were then cooled and inoculated with a 5 mm diameter disc of mycelia from PDA growing culture of *L. decastes*. Inoculated plates were incubated at 25°C for four weeks and mycelial radial extension was measured.

The effects of sterilization on mycelial growth were carried out in 100 g, 200 g, 500 g and 1kg substrate with 65% moisture content, and sterilized at 121°C for 30, 45, 60, 90 and 120 min independently. Substrate mixture, supplements, inoculation and incubation were the same as before in the experiment with moisture content. Results were recorded by visually observing them at an interval of one week for four consecutive weeks. Mycelial density (compactness of mycelia on culture media) was estimated by visual observation method.

*2.3. Effect of amino acids and vitamins*

The effects of various amino acids and vitamins were examined to find out the suitable nutrient source on mycelial extension. The basal medium contained 18 g glucose, 3 g yeast extract, 1 g polypeptone, 0.5 g $KH_2PO_4$, 0.5 g $K_2HPO_4$, 0.5 g $MgSO_4.7H_2O$, and 1000 ml distilled water, supplemented with 20 g agar as solidifying agent. Eight amino acids and six vitamins were individually supplied at 0.05% in the basal medium replacing nitrogen sources (yeast extract and polypeptone), and then it was sterilized at 121°C for 15 min. Portions (20 ml) of sterilized media were poured into the petri dishes, cooled and then inoculated with a 5 mm disc of mycelia. Mycelial colony diameter (mm) was determined after two-week incubation in darkness at 25°C. The basal medium without any nitrogen sources was used as a control.

*2.4. Effect of minerals*

Six mineral elements were individually provided at 0.1% in the basal medium excluding all other mineral sources, and were sterilized at 121°C for 15 min. Portions (20 ml) of the media was poured into the petri dishes, cooled and then inoculated with a 5 mm PDA disc of growing mycelia. It was incubated at 25°C and mycelial colony diameter (mm) was determined after a two-week incubation period in darkness. The basal medium without mineral sources served as a control.

*2.5. Statistical analysis*

All data were analyzed by one-way ANOVA (SPSS 11.5 for Windows). Tests of significant differences were determined by Tukey's B test at ($P < 0.05$).

## 3. Results and Discussion

*3.1. Effects of light*

Photoperiod showed significant effect ($p<0.05$) on the growth of fungal mycelium (Fig. 1). The highest mycelial growth was observed in total darkness, followed by 12-hour alternating shifts of dark and light. The lowest mycelial growth was found in complete light. This result contradicts with *Agaricus blazei*, which reaches maximum mycelial growth in total light condition [7]. Mycelial colony was relatively dense when it was incubated in alternating shifts of dark/light conditions; this result showed that light is also an important factor in the growth of this fungus. Though, it is interesting that the effect of light on some species appears to be localized, and it is not transferred through the mycelium to non- illuminated parts [8].

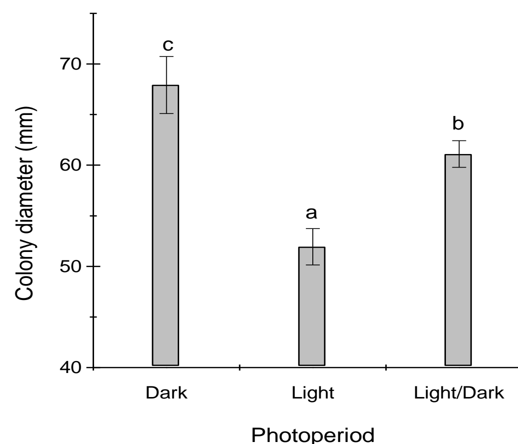


Fig. 1. Effect of photoperiod on mycelial growth. Bars represent standard deviation of the means (n = 5). Different letters at the top of the columns show significant differences at $p<0.05$.

*3.2. Effect of substrate moisture*

The moisture content of culture substrate affects mycelial growth (Fig. 2). Substrate moisture content exhibited significant increase in mycelial growth ($R^2$ = 0.98, $p$ = 0.002), and reached the highest values for 60-70%. Further increase in substrate moisture to the level of 80% showed decreasing pattern in colony diameter. Increasing the moisture level is expected to reduce the porosity of the substrate, limiting the transference of oxygen. For this reason, the use of high moisture content limited the growth within the whole substrate, resulting in surface growth [9]. The result suggests that a specific amount of water is needed to saturate the substrate for optimal growth of mycelim. Low water content, not enough to support growth, might be due to an over-dry substrate, while high moisture may result in water-logging which will not support the growth of the fungus. However, the mycelial density was rather high in the low moisture substrate, but 40% substrate moisture showed wispy mycelial growth. It is known that loss of substrate moisture should be avoided by providing high humidity during spawn run.

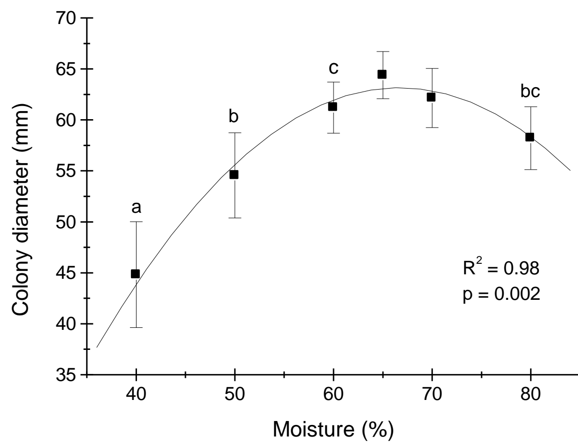


Fig. 2. Effect of substrate moisture on mycelial growth. Bars represent standard deviation of the means (n = 5). Different letters show significant differences at $p$<0.05. The fitted line shows relationship between substrate moisture and colony diameter and is based on a second order polynomial regression model.

*3.3. Effect of sterilization*

Contamination of inoculated substrate is a major problem for mushroom cultivation, resulting in considerable crop loss. The sterilization at 121 C at 30, 45 and 60 min was completely contaminated with green moulds within ten days of incubation, and 90 min sterilization was efficient up to 200 g substrate. Moreover, 500 g of substrate was efficient to reduce contamination when sterilized for 2 h. Some species grow best in fermented pasteurized substrates, while other species grow best in sterilized, pasteurized, or simply moistened substrates [10]. It is known that *L. decastes* grows best in fermented and highly sterilized media. In addition, the period of sterilization is to be increased when increasing the amount of substrate. In this study, the contamination appeared within two weeks of incubation, whereas in some cases, it appeared in a month-long incubated substrate (data not shown). Once colonized, the substrate showed no contamination. Although disinfection of fermented compost by autoclaving was successful, it is not feasible for commercial cultivation in terms of cost performance.

*3.4. Effect of amino acids and vitamins*

The effect of amino acids on the mycelial growth of *L. decastes* is presented in Fig. 3. Proline (52.86 mm) and glutamic acid (61.73 mm) showed significant increase in mycelial growth ($p$<0.05). The remaining five amino acids showed equivalent or lower response than control. Interestingly though, results also showed that as mycelial colony diameter increased, density of mycelium in the colony decreased. All the vitamins used in this study significantly ($p$<0.05) stimulated the growth of *L. decastes* (Fig. 4). Riboflavin ($B_2$) (56.57 mm) and thiamine ($B_1$) (55.90 mm) were the most stimulatory vitamin sources, as has been shown in other works [11, 12]. The remaining vitamins also had positive effects on metabolic activates for this fungus. The result of mycelial density was consistent with the result of amino acids.

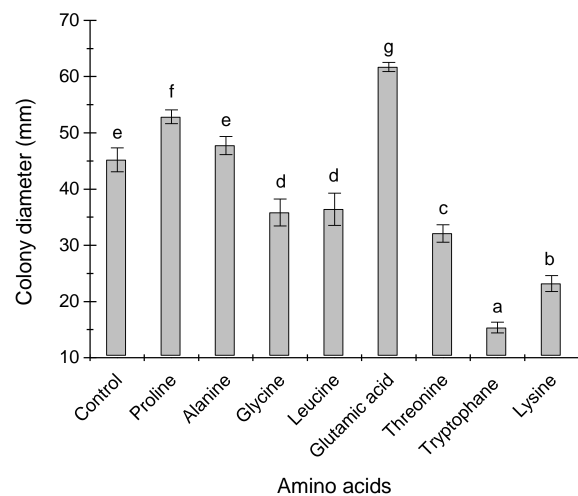


Fig. 3. Effect of amino acids on mycelial growth. Bars represent standard deviation of the means (n = 4). Different letters at the top of the columns show significant differences at $p$<0.05.

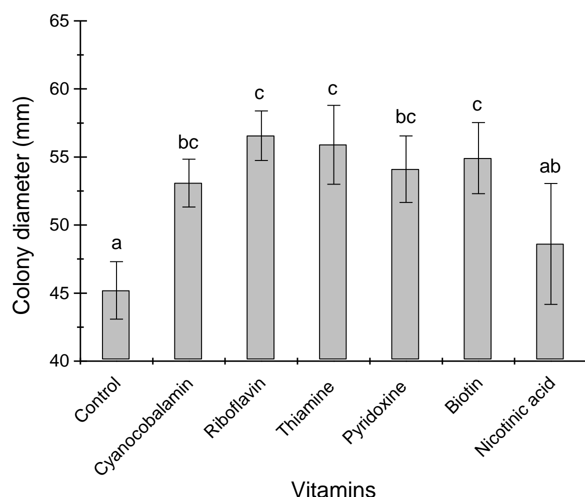


Fig. 4. Effect of vitamins on mycelial growth. Bars represent standard deviation of the means (n = 4). Different letters at the top of the columns show significant differences at $p<0.05$.

*3.5. Effect of minerals*

The effect of different minerals on mycelial growth of *L. decastes* is given in Fig. 5. Of various minerals used, $K_2HPO_4$ (66.80 mm) and $CaSO_4$ (67.78 mm) exhibited significant growth of colony diameter ($p<0.05$). This result showed that mineral nutrients are also one important factor for growth. In contrast, growth was inhibited by $MnSO_4$ and $FeSO_4$ (data not shown). Mycelial density was found higher in case of $CaSO_4$, whereas mycelial growth was lower in $CaCO_3$, with increase in density of the mycelial colony. Calcium ion plays important roles in the regulation of the growth of hyphal apices and the formation of branches, and it also could have a role as a secondary messenger [13]. Calcium is believed to act as transducer stimuli at the cell surface that may include chemical, electric or physical signals into specific intracellular effects [14].

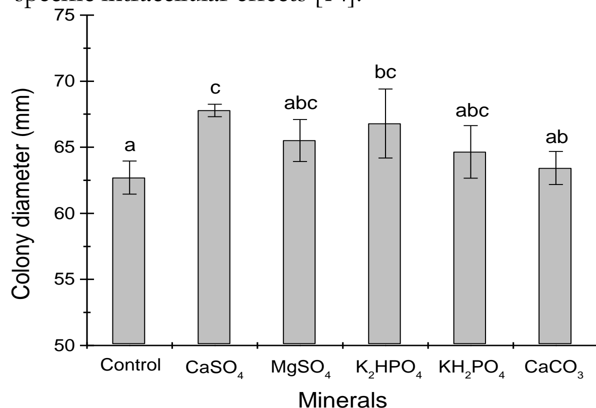


Fig. 5. Effect of mineral sources on mycelial growth. Bars represent standard deviation of the means (n = 4). Different letters at the top of the columns show significant differences at $p<0.05$.

In conclusion, a total dark condition favoured the luxuriant mycelial growth of *L. decastes*. Neither low substrate moisture condition (<50 %) nor the higher (>80 %) were suitable for growth of the fungus. Furthermore, glutamic acid and riboflavin greatly enhanced mycelial growth. The best mycelial growth was recorded from $CaSO_4$. Mineral supplements exhibited selective response towards fungal growth. Except for manganese and iron sulphates (showed no response and therefore, not given in Fig. 5), all tested minerals were very effective for enhancing the growth of mycelium. Two-hour sterilized substrate was effective in avoiding contamination. The results indicated that various factors and nutrients could be utilized to improve the growth of mycelium of *L. decastes*.

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