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## Standardization of cultural conditions *in vitro* for the maintenance of *L. Edodes* Strains

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### Abstract

Shiitake is the third most important edible mushroom and its cultivation needs to be encouraged. Presently we are lagging behind in the availability of information database regarding standardized conditions for Shiitake cultivation. So, this study was conducted to standardize the cultural conditions like medium, temperature and pH for six high temperature strains - OE-16, OE-22, OE-28, OE-38, OE-388 and Le (L) and one low temperature strain - Le (O) of *Lentinula edodes*. PDA was found to be the best medium for the vegetative growth of *L. edodes* strains. On PDA, maximum diametric growth was recorded in OE-388, Le (O) and OE-16 in comparison to least growth in OE-38. However, OE-22 and OE-28 were found intermediate in terms of their diametric growth measured. Maximum mushroom mycelium growth was achieved at  $25 \pm 1^\circ\text{C}$  and pH 4 was the best, which produced highest dry biomass of all the strains. This study revealed the optimum conditions for the maintenance of *L. edodes* culture *in vitro*

**Keywords:** Shiitake, mushroom, *Lentinula edodes*, mycelium, sporocarp, strain

### Introduction

*Lentinula edodes* (Berk.) Pegler, known as Shiitake, is the third most important edible mushroom and grown in more than 60 countries worldwide. *In vitro* mycelial growth of *L. edodes* is fluffy white with circular colony. It produces brown sporocarps with fleshy convex, light to dark brown cap, slightly tough and light brown pileus and creamy white gills, having unique flavour, aroma and taste with several nutritional and medicinal properties, that improves our immune system and protect us from cold, flu, environmental allergies, high blood pressure, diabetes and many other problems<sup>1</sup>. The production of tropical mushrooms like oyster (*Pleurotus spp.*), paddy straw (*Volvariella volvacea*) and milky mushroom (*Calocybe indica*) utilizing locally available agricultural wastes viz., paddy straw, wheat, soybean, chickpea, mustard, lathyrus, cotton wastes and lignocellulosic wastes are exploited<sup>2</sup>. But, naturally, it grows on hardwood trees but the commercial production of shiitake is successfully achieved on artificial logs composed of - lignin and cellulose rich organic matter, like saw dust, wheat straw etc<sup>3</sup>. Shiitake growing on artificial logs have problem of slow mycelium run and hard and less fruiting initiation. Keeping these facts in view, the present investigation was carried out to study the cultural behaviour, of *L. edodes* strains *in vitro* so as to standardize its growth conditions.

### Pure cultures of *L. edodes* strains and their maintenance

Seven strains of *L. edodes* (Berk.) Pegler namely OE-16, OE-22, OE-28, OE-38, OE-388, Le (L), Le (O) were selected for present study. The pure cultures of six selected *L. edodes* strains were obtained from the Mushroom Research and Training Centre (MRTC), Pantnagar, Uttarakhand, while, Le (O) (wild strain) was isolated from Veer Chandra Singh Garhwali (VCSG) College of Horticulture, Bharsar (Pauri Garhwal) Uttarakhand and the experiments were conducted in the same laboratory. All the cultures were maintained on Potato Dextrose Agar (PDA) medium (200 g peeled and sliced potato, 20 g dextrose, 20 g agar, 1000 ml distilled water) at  $25 \pm 1^\circ\text{C}$  for growth.

### Media

Effect of four different media i.e. PDA, MYPG i.e. Malt Yeast Peptone Glucose (3 g malt extract, 3 g yeast extract, 5 g peptone, 20 g glucose, 20 g agar, 1000 ml distilled water), Oat meal Agar (3 g oat meal, 0.2 g  $\text{KNO}_3$ , 0.5 g  $\text{K}_2\text{HPO}_4$ , 0.2 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 20 g agar, 1000 ml distilled water) and Czapek-Dox (2 g  $\text{NaNO}_3$ , 1 g  $\text{K}_2\text{HPO}_4$ , 0.5 g KCl, 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 g  $\text{FeSO}_4$ , 30 g sucrose, 20 g agar, 0.01 g  $\text{ZnSO}_4$ , 0.005 g  $\text{CuSO}_4$ , 1000 ml distilled water) were studied at  $25^\circ\text{C}$  for the diametric growth of seven *L. edodes* strains. The diametric length

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was measured from the centre of the inoculated disc to the outermost periphery of the mycelia thread.

### Temperatures and pH

Three incubation temperature for a week ( $21 \pm 1^\circ\text{C}$ ,  $23 \pm 1^\circ\text{C}$  and  $25 \pm 1^\circ\text{C}$ ) and different values (2.0, 3.0, 4.0 and 5.0) of the pH of the media were tested for mycelia growth (in Petri plates) on PDA medium and dry weight of mycelium in Potato Dextrose Broth (PDB) medium, respectively. The pH of the medium was adjusted with the help of pH meter using 0.1N HCl and 0.1N sodium hydroxide before sterilization (autoclaving) was done. Three replications of each treatment were maintained at  $25 \pm 1^\circ\text{C}$  and 90 rpm agitation. After 25 days of inoculation, the fungal mat and the liquid media were separated by double layered Whatman No.1 filter.

Statistical analysis of the data was done by using STPR 2 and STPR 5 with simple CRD and two factorial CRD. Critical

difference (CD) at 5% level of significance was used for comparison of difference between the treatment means.

### Results and Discussion

#### Effect of temperature on diametric growth of *L. edodes* strains in PDA

The effect of temperature on the diametric growth of *L. edodes* strains on previously selected PDA medium in which the growth of seven *L. edodes* strains was studied at temperatures  $21 \pm 1^\circ\text{C}$ ,  $23 \pm 1^\circ\text{C}$  and  $25 \pm 1^\circ\text{C}$ . Out of three temperatures tested, the temperature  $25 \pm 1^\circ\text{C}$  supported best growth of all the strains. These results were supported by scientific study<sup>4</sup>. According to them  $25^\circ\text{C}$  was an ideal temperature for *L. edodes*. At  $25 \pm 1^\circ\text{C}$  all the strains showed statistically identical growth that ranged from 8.1 to 8.5 cm and it was 5.9 cm growth in Le (O) at 11 DAI. Le (O) showed least growth rate/day than all remaining treatments (Table 1).

**Table 1:** Effect of temperature on diametric growth of *L. edodes* strains in Potato Dextrose Agar (PDA) medium

Strains	Growth at $21^\circ\text{C} \pm 1$ (cm)	Growth rate (cm/day)	Growth at $23^\circ\text{C} \pm 1$ (cm)	Growth rate (cm/day)	Growth at $25^\circ\text{C} \pm 1$ (cm)	Growth rate (cm/day)
OE-16	7.3	0.7	7.7	0.7	8.1	0.7
OE-22	8.1	0.7	8.3	0.8	8.5	0.8
OE-28	7.7	0.7	8.5	0.8	8.5	0.8
OE-38	7.4	0.7	8.1	0.7	8.1	0.7
OE-388	7.9	0.7	8.3	0.8	8.5	0.8
Le(L)	7.6	0.7	8.5	0.8	8.5	0.8
Le(O)	4.6	0.4	5.4	0.5	5.9	0.5
CD at 5 %	0.22		0.21		0.9	

#### Effect of media

PDA medium supported the vegetative growth of all strains excellently in comparison to MYPG, Oat meal and Czapek Dox medium. On PDA, maximum diametric growth i.e 8.4 cm (OE-388) to 8.5 cm (Le (O), Le (L) and OE-16) was recorded in comparison to least growth of 6.3 cm in OE-38. However, OE-22 and OE-28 were found medium in terms of

their diametric growth measured 7.7 to 7.9 cm at 15 DAI. The highest growth rate 0.6 cm /day was recorded with strain OE-388, Le (L), Le (O) and OE-16 followed by OE-22 and OE-28 in which 0.5 cm vegetative growth was recorded/day in PDA medium. However, OE-38 was inferior in case of growth rate (Table 2).

**Table 2:** Effect of different media on diametric growth of *L. edodes* strains

Strains	Potato dextrose Agar	Growth (cm/Day)	Malt yeast peptone Glucose	Growth (cm/Day)	Oat meal	Growth (cm/Day)	Czapek dox	Growth (cm/Day)
OE-16	8.4	0.6	7.9	0.5	5.9	0.4	4.2	0.3
OE-22	7.9	0.5	7.1	0.5	5.6	0.4	4.1	0.3
OE-28	7.7	0.5	7.1	0.5	5.4	0.4	4.3	0.3
OE-38	6.3	0.4	3.3	0.2	4.9	0.3	4.1	0.3
OE-388	8.5	0.6	5.6	0.4	4.9	0.3	3.9	0.3
Le(L)	8.5	0.6	6.3	0.4	4.9	0.3	4.3	0.3
Le(O)	8.5	0.6	6.3	0.4	4.7	0.3	4.1	0.3
CD at 5%	1.5		1.75		2.26		2.35	

#### Effect of pH on dry weight of mycelium of *L. edodes* strains on PDB

Among all the pH levels, pH 4 was found to produce highest dry biomass of all the strains in comparison to all corresponding strains grown at remaining pH levels. The biomass of all strains statistically varied from 0.19 to 0.3, 0.1

to 0.4, 0.36 to 0.76 and 0.14 to 0.85 g dry biomass/100 ml PDA broth at 25 DAI at pH 2.0, 3.0, 4.0 and 5.0, respectively (Table 3). The results were supported by several studies<sup>5</sup>. These studies suggested slightly acidic to neutral pH is the best for the mycelial growth of *L. edodes* strains.

**Table 3:** Effect of pH on dry weight of mycelium of *L. edodes* strains on Potato Dextrose Broth

Strains	Dry weight of mycelium of <i>L. edodes</i> strains (strains of <i>L. edodes</i> ) (g)			
	2pH	3pH	4pH	5pH
OE-16	0.19	0.36	0.38	0.34
OE-22	0.19	0.40	0.46	0.31
OE-28	0.21	0.10	0.76	0.49
OE-38	0.20	0.28	0.56	0.40
OE-388	0.24	0.24	0.54	0.14
Le(L)	0.19	0.27	0.36	0.25
Le(O)	0.31	0.32	0.70	0.85
CD at 5 %	2.45	1.32	0.41	0.55

This study shows that Potato Dextrose Agar medium, temperature range 23-25 ± 1°C and pH 4 were best suited to the mycelial growth of *L. edodes strains in-vitro*. So, the cultures of *L. edodes* can be maintained at these conditions in the laboratory.

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