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Submerged culture conditions for mycelial yield and polysaccharides production by *Lyophyllum decastes*

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Abstract

The effects of various carbon and nitrogen sources, their concentrations, initial pH and fermentation duration on the production of mycelia in terms of dry weight, exo-polysaccharide (EPS) and inner polysaccharide (IPS) by *Lyophyllum decastes,* a culinary-medicinal mushroom, were investigated in shake-flask cultures. Lactose, glucose and fructose were the top three best carbon sources for mycelial growth with corresponding yields of 6.73 g/l, 6.36 g/l and 6.10 g/l, respectively. Glucose was the best for production of EPS and IPS with 1.65 g/l and 317 mg/g dry mycelia, respectively. Maltose also performed well for EPS production. Yeast extract was the best nitrogen source for the production of mycelia (7.03 g/l) and IPS (325 mg/g dry mycelia), whereas EPS was improved further by increasing the yeast extract concentration (2.46 g/l at 2%). Similarly, initial pH 7 and 8 were best for polysaccharides production (EPS 1.73 g/l and IPS 320 mg/g) and mycelial growth (7.10 g/l), respectively. Maximum mycelial growth peaked at 15 days of cultivation whereas polysaccharides peaked at 10 days, and then tapered off. A concentration of glucose 3% and yeast extract 1% (mycelial yield and IPS) were found to be a suitable condition for submerged culture.

Keywords: *Lyophyllum decastes*; Submerged culture; Mycelial yield; Exo and inner polysaccharides

1. Introduction

Fungi are currently of interest because they are a biologically-rich source of various active substances. Polysaccharides derived from mushrooms have emerged as an important class of bio-active substances. Extracts from medicinal mushrooms have been used in traditional Oriental therapies for their antitumor and immunomodulating properties (Liu, Ooi, & Fung, 1999). Mushroom mycelial, cultivated by submerged fermentation, also have the potential to be used in the human diet (Yitzhak & Ephraim, 1986).

Lyophyllum decastes (Fr:Fr) Sing, a gray basidiomycete that belongs to the family *Tricolomataceae*, is a popular culinary-medicinal mushroom due to its good flavor and excellent texture, as well as being a well known medicinally-potent variety. 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 (Ukawa, Ito, & Hisamatsu, 2000), and the number of leukocytes, due to X-ray irradiation, was reduced by the medication of *L. decastes* (Gu et al., 2002).

Isolation and purification of polysaccharides from mushroom materials is relatively simple and straight forward, and it can be carried out with the minimal of effort (Mizuno, 1999). Most of the active polysaccharides from mushrooms form complexes with protein (Wasser, 2002). Polysaccharopeptides (PSP) is a hot water extract of mushrooms, and appears as a light or dark brown powder which is soluble and stable in hot water. The powder extract, typically contains 34–35% soluble carbohydrate (91–93% β-glucan), 28–35% protein, 0–7% moisture, 6–7% ash and the remainders are free sugars and amino acids (Ueno et al., 1980).

Physiologically-active polysaccharides can be obtained from commercially cultivated mushroom, which usually

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takes a long duration to culture due to their fruiting body. It is generally recognized that growing mushroom mycelia in a defined medium, by submerged fermentation is a rapid and alternative method to obtain fungal biomass of consistent quality (Litchfield, 1967). Furthermore, mycelial growth of mushrooms in submerged fermentation, provides a scalable production method for polysaccharides. The polysaccharides produced in submerged culture can be extracted from the mycelial biomass and biomass free culture broth. Polysaccharides isolated from different sources of mushroom, mycelium and biomass free broth, differ somewhat in structure, composition, and physiological activity. However, exo-polymer, which also exhibits similar biological effects as the polysaccharides of the mycelia, can be produced simultaneously (Kim et al., 2001).

Cultivation of *L. decastes* for the formation of fruiting body is a lengthy and complex process involving the use of fermented lignocellulose substrate. Therefore, the submerged culture method offers a feasible alternative for the production of high quality mushroom products with immuno-stimulating polysaccharides. Although, mycelial fermentations of medicinal mushrooms have often been reported, this mushroom has not been reported in submerged culture. To the best of our knowledge, this is the first report concerning the submerged culture of this fungus.

This study attempts to develop the culture conditions and media compositions necessary to produce simultaneously and efficiently, bioactive mycelia with both exo and inner polysaccharides in a submerged culture of *L. decastes*.

2. Materials and methods

2.1. Microorganism and seed culture

The KS-74 strain of *L. decastes* used in the experiment was obtained from Kyushu University. The strain was cultured on Potato Dextrose Agar (PDA) plates at 23 °C for two weeks, then maintained at 4 °C, and sub-cultured every three months.

The seed culture medium consisted of the following components: glucose 25; yeast 7; polypeptone 3, K_2HPO_4 1; KH_2PO_4 0.5; $MgSO_4 \cdot 7H_2O$ 0.5 g/l. Actively growing mycelia of *L. decastes* were transferred to the seed culture medium by punching out 5 mm of PDA with a sterilized cutter. The seed culture was grown in a 300 ml flask containing 50 ml medium and incubated at 25 °C on a rotary shaker at 125 rpm for 7 days. The basal medium was similar to the seed culture medium, but only with a glucose concentration of 30 g/l.

2.2. Shake-flask culture

The flask culture experiments were performed in a 300 ml flask containing 50 ml of the medium, and the medium pH of various nutrients was not adjusted. The flasks were stopped with silicone plugs and media were sterilized at 121 °C for 15 min. Ten percent (v/v) of the seed culture was inoculated. The fermentation was carried out in a rotary shaker incubator at 125 rpm, at 25 °C and cultured for 10 days. Different kinds of carbon and nitrogen sources and their concentration were tested. The effects of initial pH on mycelial growth and polysaccharide production were studied in different values of pH and the fermentation duration (days) were also assayed (basal medium). Initial pH (HORIBA pH METER F-21) was adjusted to the desired value by using 1 N NaOH and 1 N HCl.

2.3. Analytical methods

The exo-polysaccharide (EPS) extraction procedure was slightly modified from the method of Xu, Kim, Hwang, Choi, and Yun (2003). Cultured broths were collected from cultured shake flask and centrifuged at 10,000*g* for 20 min. The resulting supernatant was filtered through a 0.45 μm membrane filter (Millipore Ireland). The resulting culture filtrate was mixed with four volumes of 95% ethanol, stirred vigorously, and then left overnight at 4 °C. The precipitated exo-polysaccharide (EPS) was centrifuged at 10,000*g* for 20 min, and supernatant was discarded. Then, the precipitates were rewashed with ethanol and centrifuge as described as above. The precipitate of crude EPS was lyophilized and the weight of crude EPS was determined. Final pH of cultured broth was determined by using a pH meter as mentioned above.

The mycelial yield was determined after repeated washings of mycelial pellets with distilled water, then lyophilized, and the dry weight of mycelia was recorded.

The extraction procedure of crude inner polysaccharide (IPS) was slightly modified from the method of Chen, Lu, Cheng, and Wang (2005). Two hundred milligrams of lyophilized mycelia (powder) was multiply extracted with 20 ml distilled water, heated at 80 °C for 3–4 h at 100 rpm, in the shaker. The extracted slurry was cooled and filtered with a 0.45 μm membrane filter (Millipore Ireland) and concentrated in a vacuum. Four volumes of 95% ethanol was added stirred vigorously, and then allow to precipitate overnight at 4 °C. The precipitated IPS collected by centrifuging at 10,000*g* for 20 min, rewashed with ethanol and centrifuged again described as above, then lyophilized and the weight of crude IPS was estimated.

2.4. Statistical analysis

All data were analyzed by one-way ANOVA. Tests of significant differences were determined by Turkey-HSD at ($P < 0.05$). In all cases there were three replicates ($n = 3$).

3. Results and discussion

3.1. Effects of carbon sources

Carbohydrates are a major component of the cytoskeleton and an important nutritional requirement for the

growth and development of higher fungi (Xiao et al., 2006). To find suitable carbon sources for mycelial growth and polysaccharides production in *L. decastes,* seven different carbon sources were separately provided at 30 g/l in place of the carbon source (glucose) in the basal medium. The mycelial growth of this fungus occurred in a varieties of carbon sources; however, production of mycelia, exo and inner polysaccharides were quite distinct. Among the sources examined, lactose yielded the best mycelial growth (6.73 g/l). A similar result was reported in *Ganoderma lucidum* (Yajie & Zhong, 2002). Following that, the best lactose mycelial growth occurred from glucose and fructose, respectively and they did not differ significantly. Maltose and sorbitol also yielded good mycelial growth, whereas moderate growth was obtained from xylose and sucrose. Mycelial yield, final pH, EPS and IPS production in different carbon sources is given in Table 1.

The EPS production by various carbon sources ranged from 1.25 to 1.65 g/l (Table 1). Glucose (1.65 g/l) was the best carbon source for EPS production, and did not differ significantly from maltose followed by sorbitol, fructose, xylose and lactose, respectively. Minimum EPS production was attained from a sucrose medium. Our results were similar with the findings by the other investigators, who demonstrated that glucose is clearly a good carbon source for exo-polysaccharide production in submerged cultures of mushrooms (Nour El-Dein, El-Fallal, El-Shahat, & Hereher, 2004; Xu et al., 2003).

As indicated in Table 1, IPS production ranged from 187 to 317 mg/g (cell) in dry mycelia. Glucose (317 mg/g dry mycelia) was found to be the best carbon source to produce a significant increase in the IPS, followed by xylose and sorbitol. Minimum IPS was recorded from fructose. Lactose, maltose and sucrose were also well suited for IPS production though.

Similarly, various concentrations of glucose were applied for the screening of the suitability of mycelial growth and polysaccharide production. Three percent glucose was significantly desirable at achieving maximum mycelial growth and production of both EPS and IPS. Following 3% glucose, mycelial growth was best from 4%, then by 2% and 5%, respectively, with similar results for polysaccharides production (Table 2). These demonstrations clearly show that growth and other metabolic activities could happen in a specific concentration of glucose or other medium. In addition, the maximum mycelial growth accompanied by a higher production of both polysaccharides was specifically noted only in the case of glucose concentration, but not any other nutrient sources.

Glucose has been reported as a good respiratory substrate (Hammond, 1978). However, this demonstration showed that each carbon source was independently responsible in mycelial growth and polysaccharide production. Therefore, even if the same fungal species needs a different carbon source for specific metabolite production, it is possible that different carbon sources might have different effects on catabolic repression on the cellular secondary metabolism. Such a phenomenon was also claimed in submerged cultivation of several kinds of mushrooms (Hwang, Kim, Xu, Choi, & Yun, 2003; Kim et al., 2003).

3.2. Effect of nitrogen sources

Organic and inorganic nitrogen sources were investigated in order to compare mycelia growth and polysaccharides production. Eight various (1% organic and 0.1% inorganic) nitrogen sources were individually employing instead of nitrogen sources in the basal medium. Among the various nitrogen sources, yeast extract yielded the highest mycelia growth with 7.03 g/l, as well as EPS and IPS with 1.76 g/l and 325 mg/g dry mycelia, respectively. A similar result was reported in *Cordyceps jiangxiensis* (Xiao et al., 2006), and in *Lentinus subnudus* on mycelial growth (Gbolagade, Fasidi, Ajayi, & Sobowale, 2006). The stimulatory effect of yeast extract is due to its protein, amino acid, and vitamin content (Bolton & Blair, 1982). Following the yeast extract, mycelial growth was comparatively higher in organic sources such as polypeptone and peptone, respectively. Less mycelial growth was achieved from ammonium nitrate. The remaining other inorganic nitrogen sources, yielded fairly high mycelial growth. In comparison with organic nitrogen sources, inorganic nitrogen sources frequently yield relatively lower mycelial growth and EPS production in liquid cultures of mushrooms

Table 1
Effect of different carbon sources on mycelial growth and polysaccharide production by *L. decastes* in shake-flask culture

Carbon sources	Yield (g/l)	EPS (g/l)	IPS (mg/g dry mycelia)	Final pH
Glucose	6.36 ± 0.20a	1.65 ± 0.05a	317 ± 6.24a	6.12 ± 0.00
Fructose	6.10 ± 0.50a	1.35 ± 0.05bc	187 ± 3.00e	6.10 ± 0.15
Sucrose	4.30 ± 0.10c	1.25 ± 0.05c	220 ± 10.00d	7.21 ± 0.42
Maltose	5.20 ± 0.20b	1.60 ± 0.10a	220 ± 15.00d	7.25 ± 0.08
Lactose	6.73 ± 0.41a	1.30 ± 0.00bc	247 ± 2.00c	7.33 ± 0.05
Sorbitol	5.13 ± 0.30b	1.40 ± 0.00b	290 ± 10.00b	7.10 ± 0.19
Xylose	4.30 ± 0.10c	1.35 ± 0.05bc	294 ± 7.93ab	7.17 ± 0.00

Values are the mean ± SD, and alphabet letters indicate the same letters in the same column are not statistically significantly different according to Turkey-HSD ($P < 0.05$).

Table 2
Effect of different glucose concentrations on the mycelial growth and polysaccharide production by *L. decastes* in shake-flask culture

Glucose concentration (%)	Yield (g/l)	EPS (g/l)	IPS (mg/g dry mycelia)	Final pH
2	4.83 ± 0.25bc	1.33 ± 0.15b	233 ± 6.00bc	6.11 ± 0.01b
3	6.36 ± 0.20a	1.65 ± 0.05a	317 ± 6.24a	6.12 ± 0.00b
4	5.40 ± 0.52ab	1.40 ± 0.10b	242 ± 8.00b	6.19 ± 0.02a
5	4.03 ± 0.58c	1.30 ± 0.00b	218 ± 6.55c	6.20 ± 0.03a

Values are the mean ± SD, and alphabet letters indicate the same letters in the same column are not significantly different according to Turkey-HSD ($P < 0.05$).

(Yang, Haung, & Yang, 2003). However, in the case of polysaccharide production, EPS (g/l) and IPS (mg/g dry mycelium) were second best from ammonium sulphate. Polypeptone was more productive for EPS than peptone, whereas peptone for IPS. EPS was slightly higher in ammonium phosphate than peptone. From obtained results, two inorganic nitrogen sources seem to be implacable in the polysaccharide production, but they were unfavorable for mycelial growth. Table 3 shows the effect of nitrogen sources on mycelial growth, final pH, exo and endo polysaccharides production in shake-flask culture of *L. decastes*.

Various concentrations of yeast extract, 0.5%, 1%, 1.5% and 2% were applied to identify suitable concentration for mycelial growth and polysaccharides production. A yeast extract of 1% figured significantly in the stimulation of the maximum mycelial growth and inner polysaccharide production, whereas EPS production was further improved by increasing the concentration of yeast extract, yet IPS and mycelial growth declined. This indicated that increasing concentration of yeast extract had a significant effect on EPS production (2.46 g/l in 2%). Surprisingly, IPS was slightly elevated from 2% than from 1.5%, for reasons still unclear. This result was not consistent with the result of glucose concentration and the changing final pH of broth also in opposite manner. Table 4 shows the effect of yeast extract concentrations on mycelial growth, final pH and polysaccharide production.

Mycelial growth appeared to be stimulated by all organic sources. Relative to the organic nitrogen sources, the inorganic nitrogen sources were not efficient for mycelial growth, whereas polysaccharide production improved greatly. In general, good mycelial growth does not seem to be a determining factor for a high production of polysaccharides; however, this finding may not be applicable in the case of glucose concentration. A similar result was claimed in *Antrodia cinnamomea* (Lin & Sung, 2005), and *Pleurotus pulmonarius* (Nour El-Dein et al., 2004).

3.3. Effect of initial pH

L. decastes was cultivated in the basal medium with different values of initial pH (4.0–9.0). Table 5 shows the effects of initial pH on mycelial yield, final pH and polysaccharide production. The pH of the culture medium is a vital factor that governs mycelial growth and polysaccharide production. The pH of the medium is very important but it is often a neglected environmental factor (Kim et al., 2005). Many investigators claimed that the different morphology of fungi mycelia under a different initial pH value was the critical factor in biomass accumulation and metabolite formation (Shu & Lung, 2004; Wang & McNeil, 1995). The medium pH may affect cell membrane function, cell morphology and structure, the uptake of various nutrients, and product biosynthesis (Gerlach et al., 1998; Shu & Lung, 2004). The mycelial growth and polysaccharide production were significantly affected by culture pH of *L. decastes*. The optimal initial pH for mycelial growth was 8 with mycelial

Table 4
Effect of different yeast extract concentrations on the mycelial growth and polysaccharide production by *L. decastes* in shake-flask culture

Yeast extract concentration (%)	Yield (g/l)	EPS (g/l)	IPS (mg/g dry mycelia)	Final pH
0.5	4.33 ± 0.25b	1.50 ± 0.10c	291 ± 16.52b	6.43 ± 0.03a
1	7.03 ± 0.20a	1.76 ± 0.05b	325 ± 6.24a	6.39 ± 0.02ab
1.5	6.90 ± 1.01a	1.90 ± 0.10b	282 ± 12.12b	6.31 ± 0.01ab
2	6.60 ± 0.20a	2.46 ± 0.05a	305 ± 5.00ab	6.29 ± 0.08b

Values are the mean ± SD, and alphabet letters indicate the same letters in the same column are not significantly different according to Turkey-HSD ($P < 0.05$).

Table 5
Effect of initial pH on the mycelial growth and polysaccharide production by *L. decastes* in shake-flask culture

Initial pH	Yield (g/l)	EPS (g/l)	IPS (mg/g dry mycelia)	Final pH
5	5.40 ± 0.20d	0.96 ± 0.05d	125 ± 5.00c	4.82 ± 0.04e
6	5.90 ± 0.10c	1.30 ± 0.10bc	225 ± 10.00b	5.57 ± 0.00d
7	6.60 ± 0.20b	1.73 ± 0.20a	320 ± 9.16a	6.18 ± 0.01c
8	7.10 ± 0.10a	1.55 ± 0.05ab	148 ± 10.81c	6.66 ± 0.01b
9	3.36 ± 0.15e	1.25 ± 0.05cd	130 ± 11.78c	7.10 ± 0.10a

Values are the mean ± SD, and alphabet letters indicate the same letters in the same column are not significantly different according to Turkey-HSD ($P < 0.05$).

Table 3
Effect of different nitrogen sources on the mycelial growth and polysaccharide production by *L. decastes* in shake-flask culture

Nitrogen sources	Yield (g/l)	EPS (g/l)	IPS (mg/g dry mycelia)	Final pH
Yeast extract	7.03 ± 0.20a	1.76 ± 0.05a	325 ± 6.24a	6.39 ± 0.02
Peptone	4.60 ± 0.20c	0.96 ± 0.05cd	268 ± 2.64b	6.15 ± 0.00
Polypeptone	6.26 ± 0.10b	1.20 ± 0.10bc	215 ± 13.22c	5.66 ± 0.00
Ammonium sulphate	2.90 ± 0.10e	1.30 ± 0.10b	277 ± 3.60b	5.58 ± 0.07
Ammonium phosphate	2.50 ± 0.10ef	1.10 ± 0.10bc	268 ± 4.35b	6.33 ± 0.08
Ammonium tartrate	3.33 ± 0.11d	0.56 ± 0.05e	151 ± 5.29e	5.07 ± 0.04
Ammonium chloride	2.63 ± 0.05ef	0.80 ± 0.10de	192 ± 7.54d	4.85 ± 0.00
Ammonium nitrate	2.43 ± 0.05f	0.70 ± 0.10e	175 ± 5.00d	4.99 ± 0.01

Values are the mean ± SD, and alphabet letters indicate the same letters in the same column are not significantly different according to Turkey-HSD ($P < 0.05$).

yield 7.10 g/l, whereas EPS and IPS achieved maximum peak at pH 7, with a corresponding 1.73 g/l and 320 mg/g dry mycelium, respectively. After pH 7, the maximum of EPS and IPS was achieved from 8 and 6, respectively. Mycelial yield was significantly lower from pH 9. Similarly EPS and IPS were from pH 5, whereas growth did not thrive at pH 4. This result gave some information about changing the balance between cell production and metabolic product. It has been reported that several kinds of mushroom have more acidic pH optima for mycelial biomass and EPS accumulation during their submerged cultures (Kim et al., 2003; Lee, Park, & Park, 1989; Shu & Lung, 2004). This mushroom did not perform the same as these findings. Interestingly, with regards to pH only, a suitable pH was neutral and a slightly alkaline medium for maximum production of polysaccharides and mycelia, respectively.

3.4. Effect of fermentation duration

The mycelial growth was increased onwards by 15 days of cultivation. Maximum growth was achieved at 6.90 g/l on cultivation day 15. This did not differ significantly from day 10 (6.60 g/l). In general, the harvest time of the mycelium, in complex carbohydrate medium should not extend beyond 20 days after the inoculation in order to avoid fungal cell lysis (Wu, Cheung, Wong, & Huang, 2003). Similarly, a decrease in yield in this fungus on day 20 may have been caused by fungal cell lysis. Polysaccharide production peaked on day 10 and then significantly decreased by day 15, yet EPS and IPS increased again somewhat by day 20. The final pH value of cultured broth dropped to 4.85 on day 15 from pH 7 at the beginning. Thereafter, it increased slightly on day 20. A similar tendency of changing the final pH of culture broth was also reported in *Cordyceps pruinosa* (Xiao et al., 2004). Minimum pH value and maximum mycelial yield, as well re-bound in final pH value and polysaccharide production, showed some systematic co-relation between final pH, mycelial growth and polysaccharide production. Comparing before and after 10-day cultivations, both EPS and IPS were much higher in earlier (on day 5) than after 10-day cultivations, yet mycelial yield was enhanced in the later rather than the earlier. This result showed that the activity of polysaccharide production was rather short-phased, even though mycelial growth continues for a longer duration. Table 6 shows the effects of fermentation periods on mycelial yield, final pH and polysaccharides production.

This result implies that the highest mycelia yield and polysaccharide production, changes with cultivation time periods. Appropriate harvest time selection is also an important factor to obtain the maximum fungal production in submerged culture. There was a time difference for better production of mycelia and polysaccharides, although, day 10 was the suitable termination day for submerged culture of *L. decastes*.

In this paper, isolated crude IPS was expressed by mg/g dry (lyophilized) mycelia. If it were expressed in g/l, relatively glucose, lactose and sorbitol enhanced the IPS production (g/l) higher than EPS and the rest of the carbon sources did not. Yeast extract, polypeptone and peptone were rather progressive nitrogen sources for IPS production than EPS, and the remaining nitrogen sources were not. Similarly, pH 7 and 6, as well as day 10, improved the inner polysaccharide production. However, production of ISP per gram dry mycelia was quite satisfactory from all nutrients sources and conditions. IPS production of *L. decastes* increased proportionally with an increasing mycelial growth from culture. Hence, maximum production of mycelial containing higher polysaccharide is one of the factors of maximization of IPS.

Table 6
Effect of cultivation days on the mycelial growth and polysaccharide production by *L. decastes* in shake-flask culture

Cultivation days	Yield (g/l)	EPS (g/l)	IPS (mg/g dry mycelia)	Final pH
5	2.70 ± 0.20b	1.40 ± 0.00b	215 ± 11.78b	6.42 ± 0.03a
10	6.60 ± 0.20a	1.73 ± 0.20a	320 ± 9.16a	6.23 ± 0.02b
15	6.90 ± 0.36a	1.25 ± 0.05b	180 ± 13.22c	4.81 ± 0.13d
20	6.30 ± 0.30a	1.35 ± 0.05b	202 ± 15.52bc	5.12 ± 0.00c

Values are the mean ± SD, and alphabet letters indicate the same letters in the same column are not significantly different according to Turkey-HSD ($P < 0.05$).

The crude IPS production from mushroom fruit bodies of *L. decastes* produced by various supplemented agriculture waste, were significantly lower (Pokhrel & Ohga, 2007), by comparison to mycelial IPS production. Our results were similar to the reports for *Inonotus obliquus* (Kim et al., 2005).

Each and every claim makes interesting results for the growth and polysaccharide production. Therefore, a selection of a definite protocol is crucial. In fact, it has been successfully applied to the improvement of the culture media for the production of primary and secondary metabolites on the fermentation process. In the present study, production of the mycelial yield and bio active polysaccharides were reasonably comparable to current practices on various mushrooms. Moreover, this work also opens a door for further studies in order to attain even greater production of mycelia and polysaccharides of *L. decastes*.

4. Conclusions

This fungus (*L. decastes*) could be effectively cultivated in submerged culture for the production of both EPS and IPS. To date, no reports are available in the literature regarding the fermentation conditions for mycelial growth, EPS and IPS production of *L. decastes*. Therefore, our study solely concentrated on selecting a suitable nutrient source, their concentration, initial pH and fermentation duration so as to maximize the mycelial yield and polysaccharide production. However, the use of an appropriate application of nutrient sources and growth parameters, are the significant factors in mycelial growth and polysac-

charide production. Further investigation on the optimization of submerged culture to achieve the demands of large scale mycelia and polysaccharides, are under our study aims using various parameters.

The growth of this mushroom in substrate, is a lengthy and complex process involving the use of fermented lignocellulosic wastes followed by a long cultivation period. In contrast, submerged culture obviously gives rise to potential advantages of higher mycelial yield and bioactive polysaccharide production in a compact space and shorter time without significant problems of contamination.

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