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Nutrient Requirements and Fermentation Conditions for Mycelia and Crude Exo-Polysaccharides Production by *Lentinus squarrosulus*

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Abstract

***Lentinus squarrosulus* Mont. is an emerging tropical white rot basidiomycete, with nutritional and medicinal benefits. Low levels of commercial cultivation of the mushrooms limit their availability for use as food and medicine. Mycelia from submerged fermentation are a suitable alternative to the mushroom from *L. squarrosulus*. Three strains, 340, 339 and 218, were studied to determine optimum growth conditions for mycelia mass and crude exo-polysaccharides (CEPS) production. The experiments were conducted in a completely randomized design (CRD) with a factorial structure. Nutrients involving 8 carbon and 8 nitrogen sources were screened, and concentrations of the best sources were optimized. Optimized nutrients, interaction between strains and other parameters such as agitation and medium volume were investigated to obtain optimum fermentation conditions for biomass and CEPS production. Biomass yield varied among strains depending on carbon or nitrogen nutrient sources. Starch and yeast extract at 30 and 25 g/L were identified as the most important nutrients in mycelia and CEPS production. Nutrient optimization resulted in a 3-fold increase in mycelia mass: 12.8, 10.0 and 15.3 g/L in strains 340, 339 and 218 respectively. There was a significant interaction between strain, agitation, and volume ($p < 0.001$). Mycelia mass increased with volume under shake conditions, while polysaccharides decreased. There was a weak and negative correlation between mycelia mass and polysaccharides ($p = 0.02$). Static conditions favored more polysaccharide production. Optimized fermentation conditions resulted in very high increase in biomass: 238.1, 266.9 and 185.0 g/L in strains 340, 339 and 218 respectively. Results obtained could be useful in modeling fermentation systems for large-scale production of mycelia mass, CEPS and other bio-products from *L. squarrosulus*.**

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Keywords

Mycelia Mass, Carbon and Nitrogen Requirements, Exopolysaccharides, *Lentinus squarrosulus*, Submerged Fermentation

1. Introduction

Lentinus squarrosulus Mont. is a white-rot basidiomycete found in many countries in African and Asia. Its fruit bodies are consumed as food in Sub-Saharan Africa and Southeast Asia [1]. The fruit body, if harvested within 3 days of fruiting, is used as a meat substitute. It was not until recently that research started to emerge on its application in food [2], medicine [3] [4] and bioremediation [5]. Proximate analysis shows that both the cap and stipe are rich in proteins, sugars, fiber, lipids, amino acids, vitamins B, C, and D, and minerals [6]. The water soluble glucans from *L. squarrosulus* have immune-enhancing properties [7], while water soluble extract from mycelia eliminated ulcer in rats within 72 hours [6]. Other investigators reported its potential as a biocontrol agent against *Rigidoporus lignosus*, a fungal pathogen of rubber, and an antimicrobial against *Bacillus subtilis*, *Mucor ramannianus* and yeast [4].

Submerged fermentation has been used widely in the production of mycelia and bioactive compounds in other basidiomycetes [8]-[10]. The process offers several advantages including: high productivity, compact and controlled environment for quality and consistency of products, and shortened production time [8]. Therefore it is reasonable to explore some factors that could enhance mycelia yield and CEPS secretion in *L. squarrosulus.* Nutritional and physiological factors are critical for bioprocess optimization during fermentation [11]. However, there is scant information on how these factors affect mycelia yield and crude exopolysaccharide (CEPS) secretions in *L. squarrosulus*. Carbon and nitrogen as nutrients are identified to be essential for cell survival, and can greatly influence cell proliferation, metabolite biosynthesis and secretion in submerged fermentation [12]. Variability in utilization of different carbon sources by basidiomycetes within the same species and across genera is widely reported [13]. Therefore it was necessary to include various sources of these nutrients. Eight different commonly used and affordable carbon and nitrogen sources were selected and tested. Since basidiomycetes require oxygen for aerobic respiration, agitation was necessary for homogenous distribution of available oxygen [12] [14].

To date there are only two reports on the effect of nutrient factors on vegetative mycelia growth in *L. squarrosulus* [15] [16]. The authors studied the effect of medium components on only one strain, but did not consider agitation and scalability. The present study investigates the influence of different sources and concentrations of carbon and nitrogen, as well as agitation, on mycelia growth and CEPS secretion in three strains of *L. squarrosulus.* The study also presents data on optimized medium for the growth of *L. squarrosulus* under static and shake fermentation conditions. Furthermore, the optimized conditions were studied for applicability in scale up fermentation.

2. Methods

2.1. Microorganisms and Inocula

Three strains of *L. squarrosulus*, 340, 339 and 218, used in the study were from the culture collection of the Mushroom Biology and Fungal Biotechnology Laboratory at North Carolina A&T State University in Greensboro. Strain 218 originated from Ghana, while strains 339 and 340 are from Okinawa Japan. The strains were maintained on PDA slants at 4˚C until used. The cultures were activated by subculturing unto PDA media and incubated at 30˚C for 3 days. Inocula for all experiments were prepared by blending a plate culture (60 × 15 mm) of each strain with 200 ml of sterile distilled, de-ionized water, using a Warring blender. All experimental media were seeded with 2% v/v inoculum.

Experiments were conducted using a basal medium composed of the following compounds, per liter: KH_2PO_4, 1 g; NaH_2PO_4, 0.4 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; $CuSO{\cdot}7H_2O$, 0.5 g; $CaCl_2{\cdot}H_2O$, 74 mg; $ZnSO_4{\cdot}7H_2O$, 6 mg; $FeSO_4{\cdot}7H_2O$, 5 mg; $MnSO_4{\cdot}4H_2O$, 3.79 mg; $CoCl_2{\cdot}6H_2O$, 1 mg; Thiamine HCl, 0.1 mg; Pyridoxine HCl, 0.1 mg; Nicotinic acid, 0.1 mg. Carbon and nitrogen sources, and their concentrations, varied according to the parameter tested. The media were sterilized at 121˚C for 15 min except for the fructose-containing media, which were au-

toclaved at 110˚C.

2.2. Nutrient Requirements for Mycelia Production

2.2.1. Carbon Sources

To determine the best carbon source for optimum yield of mycelia, a 3 × 8 factorial experiment was set up in 50 mL basal medium (in 250 ml Erlenmeyer flasks) containing 10 g/L of one of the following carbon sources: dextrose, fructose, mannose, mannitol, sorbitol, sucrose, starch or xylose. The control medium had no sugar. Each treatment flask was separately inoculated with either strain 340, 339 or 218 and each treatment was replicated five times. Inoculated media were incubated at 30˚C for 14 days. Mycelia biomass were harvested by filtration through Whatman No. 1 filter paper, washed three times and dried to a constant weight at 65˚C.

2.2.2. Nitrogen Sources

To determine the best nitrogen source for mycelia yield, a 3 × 8 factorial experiment (strain and nitrogen source) was set up. Eight grams per liter of organic nitrogen sources—peptone, yeast extract, corn steep liquor and urea, and nitrogen contents corresponding to 0.96 g/L from inorganic sources—potassium sulfate, ammonium nitrate and ammonium sulfate—were separately used in the growth medium. Starch (10 g/L) identified from previous experiments was used as the carbon source. Sterilization, inoculation, incubation and mycelia yield determination were done as described in Section 2.2.1.

2.2.3. Concentrations of Selected Carbon and Nitrogen Sources

To determine the most appropriate concentration of selected carbon (starch) and nitrogen (yeast extract) sources to support high mycelia yield, two separate 3 × 9 factorial experiments of strain and starch or yeast extract was conducted. The strains were cultivated at different concentrations ranging from 0 - 30 g/L of starch or yeast extract. Mycelia was harvested and determined as described in Section 2.2.1.

2.3. Scale up Fermentation under Shake and Static Conditions for Mycelia and Crude Exopolysaccharide Production

Series of 3 × 5 × 2 experiments were conducted to determine the effect of strain, volume and agitation on mycelia and exopolyssacharide yield. The strains were incubated for six days in an optimized medium composed of starch at 30 g/L, and yeast extract at 25 g/L in 5 different scale-up medium volumes of 50, 100, 250, 500, and 1000 mL under static and shake ~150 rpm (Barnstead/Lab-Line Max Q4000) conditions. Mycelia was harvested by filtration with Whatman no. 1 filter paper and washed three times. Crude exopolysaccharide (CEPS) was precipitated as previously reported [17]. The culture filtrates were stirred with 4 volumes of absolute ethanol, mixed vigorously and stored at 4˚C overnight. Precipitated CEPS were pelleted at 10,000 g (Eppendorf Centrifuge 5430R) at 4˚C for 10 min. Harvested mycelia and CEPS were dried at 65˚C to constant weight. Mycelia mass and CEPS were expressed as g dry weight/L of culture liquid.

2.4. Experimental Design and Statistical Analysis

The experimental design is a completely randomized design (CRD) with 2 or 3 factorial structure depending on the experiment. All experiments were carried out in 5 replicates, and the results are expressed as mean values. Differences among means were compared using Duncan's multiple range test. Statistical Analysis Software (SAS) version 9.3 was used for analysis of variance (ANOVA). Mean comparisons, and regression and correlation analysis were carried out where applicable. Under scale-up fermentation experiments, analysis of variance was performed to determine whether there is main effect or interaction between any two or all of the variables in the 3 factors tested. Regression and correlation analysis was used to estimate the variability in mycelia/polysaccharide yield due to culture volume, while correlation measured the strength of the relationship between mycelia yield and polysaccharide secretion among strains.

3. Results and Discussion

3.1. Utilization of Different Carbon Sources

The effect of eight different carbon sources on mycelia growth in strains 340, 339 and 218 of *L. squarrosulus*

was studied. Analysis of variance (ANOVA) shows that there is a highly significant interaction between strain and carbon source ($p < 0.0001$). Mean comparison using Duncan's multiple range test shows that mycelia yield was highest when starch and mannose were used as carbon sources regardless of strain (**Table 1**). In these two carbon sources, strains 340 and 218 had similar mean mycelia yield of 4.31 g/L that was significantly different from strain 339 (3.29 g/L). Beyond these two sugars, strains vary significantly in their growth response to other carbon sources studied. Modest mycelia yield was achieved with dextrose only in strain 218. The sugar alcohols (mannitol and sorbitol) produced higher mycelia mass in strain 218 than strains 339 and 340. The least mycelia yield was produced with fructose, xylose and sorbitol in strains 218, 339 and 340 respectively. The result from the study is consistent with other work that reported starch and mannose as being widely utilized by many basidiomycetes [10] [18]. Starch utilization is possible because some basidiomycetes synthesize effective amylolytic enzymes for hydrolysis of starch. Literature shows that mannose and dextrose are good substrates for cellular respiration [19]. Therefore, it is not surprising that the strains studied produced high biomass with mannose. Similar response is seen in other basidiomycetes including *Cordycceps militaris* [18], *Pleurotus tuber-regium* [20], and *Grifola frondosa* [21]. Intermediate utilization of mannitol by strain 218 and other basidiomycetes is associated with substrate oxidation or dehydrogenase enzyme activity [22]. In contrast, Gbolagade *et al*. [15] reported that other sugars such as fructose and maltose stimulated the most growth in *L. subnudus* (syn. *Lentinus squarrosulus*). The highest mean mycelia mass reported in their work was remarkably lower, 0.19 g/L, compared to 4.42 g/L observed in the present study. Since mean mycelia yield did not differ between starch and mannose within the strains studied, starch was used in further experiments, since it is more readily available and cheap compared to mannose.

3.2. Utilization of Different Nitrogen Sources

Analysis of variance shows that there is interaction between nitrogen source and strain ($p < 0.001$), resulting in differences in mycelia yield among strains (**Table 2**). Organic nitrogen sources were generally preferred over inorganic sources except urea. Strain 218 did not grow on urea supplemented medium. Among organic sources, yeast extract was clearly superior to peptone and corn steep liquor ($p < 0.0001$). With yeast extract there was no significant difference between strains 340 and 218, which had higher mean mycelia yield of 6.17 g/L compared to 5.61 g/L in strain 339 ($p < 0.0001$). Mycelia yield was significantly reduced with inorganic nitrogen sources. KNO_2 and the control treatments inhibited growth of all strains. The preference of basidiomycetes for organic nitrogen sources has been reported by others in experiments with *Lentinus subnudus* [15], *Agaricus cinnamomea* [23], and *Hericium erinaceus* [24]. Most basidiomycetes prefer complex organic nitrogen sources in submerged fermentations, probably because certain essential amino acid(s) are not readily synthesized from inorganic sources during fermentation [25]. The result is supported by Jennison *et al*. [26], who reported that white and brown rot fungi failed to grow when potassium nitrate, potassium nitrite, and ammonium chloride were used as nitrogen sources. Growth was significantly higher with ammonium nitrate than ammonium sulfate ($p < 0.0001$).

Table 1. Mycelia yield (g/L) of three strains of *L. squarrosulus* grown in 8 different carbohydrate sources.

Carbon sources	Strain 218	Strain 339	Strain 340
	Mycelia dry weight g/L*		
Starch	4.30^{a}	3.30^{bcd}	4.39^{a}
Mannose	4.42^{a}	3.27^{cd}	4.30^{a}
Dextrose	4.30^{a}	2.48^{fgh}	3.54^{bc}
Mannitol	2.82^{def}	1.47^{kl}	1.86^{ijk}
Sorbitol	2.65^{efg}	1.35^{klm}	1.73^{ijkl}
Xylose	2.00^{hij}	1.07^{m}	3.36^{bcd}
Fructose	1.604^{jklm}	1.82^{ijk}	3.06^{cde}
Sucrose	2.00^{hij}	2.24^{ghi}	3.85^{ab}
Control	1.24^{lm}	1.07^{lm}	1.24^{lm}

*Means with the same letters within columns and rows are not significantly different ($p < 0.0001$).

Table 2. Mycelia yield (g/L) of 3 strains of *L. squarrosulus* grown in 8 different nitrogen sources.

Nitrogen sources	Strain 218	Strain 339	Strain 340
	Mycelia dry weight g/L*		
		Organic	
Yeast Extract	6.12^{a}	5.61^{b}	6.22^{a}
Peptone	5.24^{b}	4.47^{c}	5.24^{b}
Corn Steep Liquor	2.85^{de}	3.02^{d}	2.59^{e}
Urea	0.63^{i}	0.95^{hi}	1.06^{gh}
		Inorganic	
$(NH_4)_2SO_4$	2.17^{f}	0.57^{i}	2.07^{f}
NH_4NO_3	1.35^{g}	0.91^{j}	1.38^{g}
KNO_3	0.057^{j}	0.07^{hi}	0.06^{j}
KNO_2	0.00^{j}	0.00^{j}	0.00^{j}
Control	0.00^{j}	0.00^{j}	0.00^{j}

*Means with the same letters within columns and rows are not significantly different ($p < 0.0001$).

The apparent differences in utilization of various ammonium compounds could arise in part from differences in hydrogen ion concentrations produced in aqueous solutions by the compounds. Ammonium sulfate supplies twice the acidity as ammonium nitrate in solution. Mean mycelia yields from yeast extract was significant among strains, and therefore it was used in further studies.

3.3. Effect of Different Concentrations of Selected Carbon and Nitrogen Sources on Mycelia Yield

In previous experiments, starch and yeast extract were selected as the best carbon and nitrogen sources that support cell growth and high biomass yield in the strains studied. It was important to optimize the concentration of these nutrients in order to maximize mycelia yield. ANOVA shows that strain and concentration interact with both nutrients at a highly significant level ($p < 0.0001$). Regression analysis shows that there is a non-linear relationship between starch or yeast extract concentration and mycelia yield; the equation of the model is displayed in **Figure 1** and **Figure 2** respectively. The strains had variable yield in mycelia ($p < 0.05$) depending on concentration of starch or nitrogen within the range tested. Starch accounted for more variability in mycelia yield than yeast extract in strains 218 (96% versus 87%) and 339 (95% versus 79%) while they seem to be of equal strength in strain 340 (84% versus 85%). Mean mycelia yield was highest at elevated starch concentrations of between 20 - 30 g/L. Strains 218 and 340 produced the most mycelia at 30 g/L, although there was no difference in mycelia yield between 25 and 30 g/L in strain 339 ($p = 0.02$). Based on statistical data, 30 g/L of starch was selected and used in further experiments.

Strain response to yeast extract is different from starch. Strain 218 and 340 produced the most mycelia with no difference in mean yield between 20, 25 and 30 g/L yeast extract. Therefore 25 g/L was selected as the median concentration for further research. Biomass was significantly lower ($p = 0.05$) in strain 339 compared to others, however its response to yeast extract concentration was similar to other strains.

3.4. Scale up Fermentation for Mycelia Yield and Crude Exopolysaccharide (CEPS) Production in Optimized Medium under Static and Shaken Conditions

The result (**Table 3**) from Analysis of Variance (ANOVA) shows that there is highly significant interaction ($p < 0.0001$) in all possible combinations of the three factors tested (strain versus agitation, strain versus volume, volume versus agitation, strain versus volume versus agitation). Since the factors considered particularly influence fermentation, data interpretation follows a 3-way ANOVA. Regardless of strain type and culture volume, shake

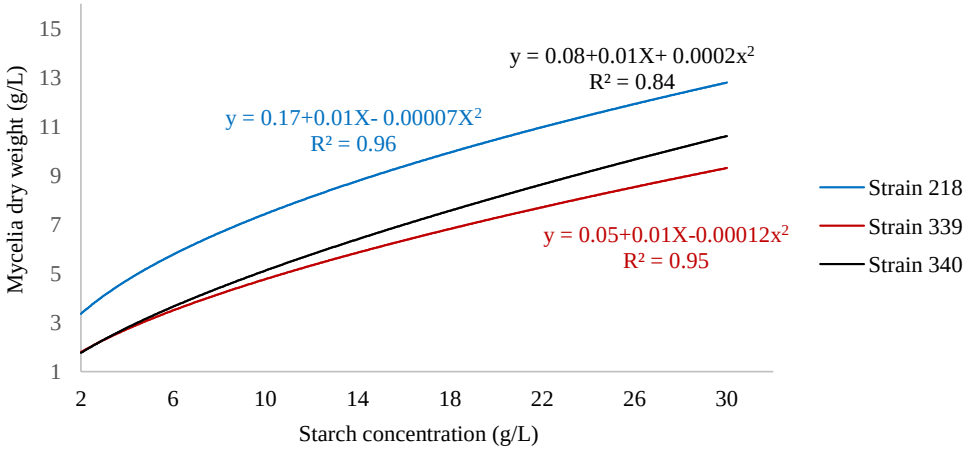

Figure 1. Regression of mycelia yield in 3 strains of *L. squarrosulus* cultured in different concentrations of starch as sole carbon source.

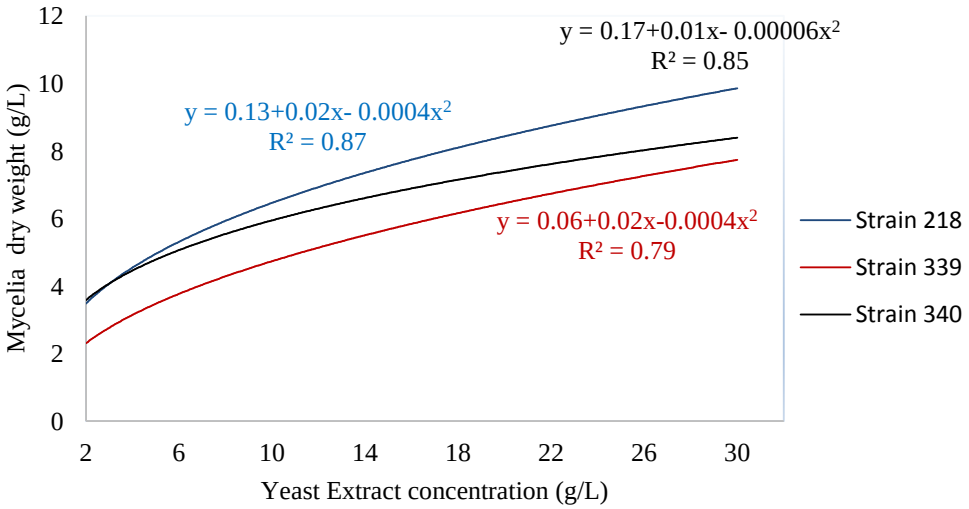

Figure 2. Regression of Mycelia yield in 3 strains of *L. squarrosulus* cultured in different concentrations of yeast extract as solenitrogen source.

Table 3. Analysis of variance of mycelia yield in 3 strains of *L. squarrosulus* showing interaction between 3 factors (strain, volume and agitation).

Sources of variation	df	Sum of squares	Mean square	F value	Pr > F
Treatment	29	682,495.72	23534.33	555.60	<0.0001
Strain	2	5099.89	2549.94	60.20	<0.0001
Volume	4	215,214.55	53,803.63	1270.20	<0.0001
Strain × volume	8	8747.07	1093.38	25.81	<0.0001
Agitation	1	214,880.50	5072.90	142.32	<0.0001
Strain × agitation	2	3134.04	1567.02	36.99	<0.0001
Volume × agitation	4	228,147.83	57,036.96	1346.53	<0.0001
Strain × volume × agitation	8	7271.82	908.97	21.46	<0.0001
Error	120	5083.02	42.36		
Total	149	687,578.75			

fermentation resulted in a highly significant increase in mycelia mass ($p < 0.0001$) than static fermentation (**Table 4**). The difference in mycelia yield under the two conditions ranged from 19.45 - 255 g/L, depending on volume of culture medium. Under agitation, mycelia mass increased with volume, whereas the reverse was observed with static fermentation, and mycelia mass decreased with volume. This pattern is consistent in the three strains tested. In similar observations, *Cordyceps jianxiensis* had a reduced growth when the culture volume increased from 50 to 300 mL under static fermentation condition [27]. In all strains, the order of increase in mycelia yield was 50 mL < 100 mL < 250 mL < 500 mL <1000 mL. The three strains produced the most mycelia mass in the highest volume tested (1000 mL). At this volume, mycelia mass produced by strain 339 (266 g/L) is higher and differs significantly from strain 340 (239.8 g/L) and strain 218 ((185 g/L) (**Table 4**). These values are higher than 25.8 g/L reported by Ahmad *et al*. [16]. Regression analysis of mycelia yield as a function of volume (**Figure 2**), was significant with an R^2 of 0.29. Since 29% of variability in mycelia mass is explained by volume, it is possible that other parameters such as nutrient and agitation may have played major roles in influencing mycelia yield. There is a highly significant interaction ($p < 0.0001$) between strain, volume and agitation in crude exopolysaccharide (CEPS) secretion. The influence of agitation on CEPS production in submerged fermentation by other mushroom have been reported [28] [29]). High mycelia biomass did not necessarily lead to high CEPS secretion in strains studied (**Figure 3** and **Figure 4**). In fact, CEPS secretion decreased with increase in mycelia yield. This is consistent with the work of Nour El-Dein *et al*. [30] on *Pleurotus pulmonarius*, Lin and Sung [31] on *Antrodia cinnamomea*, Isikhuemhen *et al*. [17] on *L. squarrosulus* under Solid State Fermentation (SSF) and Diamantopoulou *et al*. [32] [33].

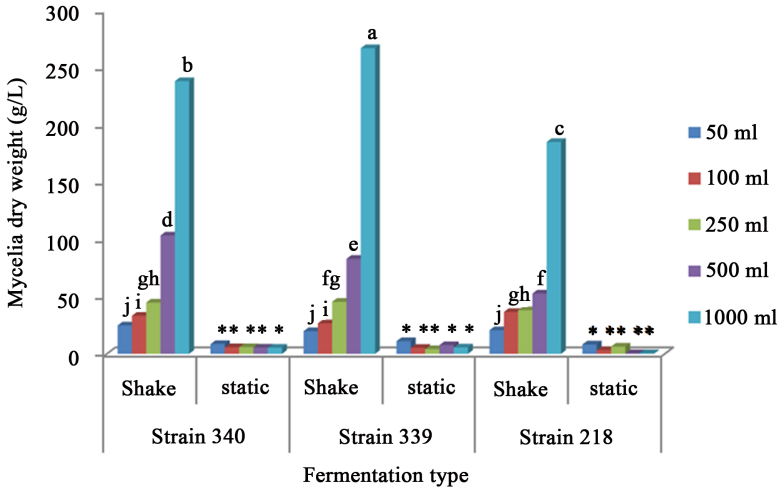

Figure 3. Mycelia dry weight (g/L) of 3 strains of *L. squarrosulus* grown in 5 different volumes of optimized medium (50 - 1000 mL) with or without agitation. Bars with same letter or symbol (*) indicate that the means are not significantly different ($p < 0.0001$).

Table 4. Mycelia dry weight resulting from submerged fermentation in 3 strains of *L. squarrosulus* using optimized medium, at different volumes with or without agitation.

Volume (mL)	Shake			Static		
	Strain 218	Strain 339	Strain 340	Strain 218	Strain 339	Strain 340
	Mycelia dry weight g/L*					
50	20.60^{K}	19.97^{K}	24.66^{K}	8.18^{lm}	11.02^{l}	8.50^{lm}
100	36.50^{HI}	26.90^{JK}	$33.4I^{J}$	3.21^{lm}	5.33^{lm}	5.78^{lm}
250	38.06^{ghi}	45.42^{fg}	44.76^{fgh}	6.30^{lm}	4.14^{lm}	5.78^{lm}
500	52.63^{f}	82.71^{e}	103.32^{d}	0.60^{m}	7.59^{lm}	5.47^{lm}
1000	185.00^{c}	266.08^{a}	238.80^{b}	0.53^{m}	5.60^{lm}	5.34^{lm}

*Means with same letters are not significantly different ($p < 0.0001$).

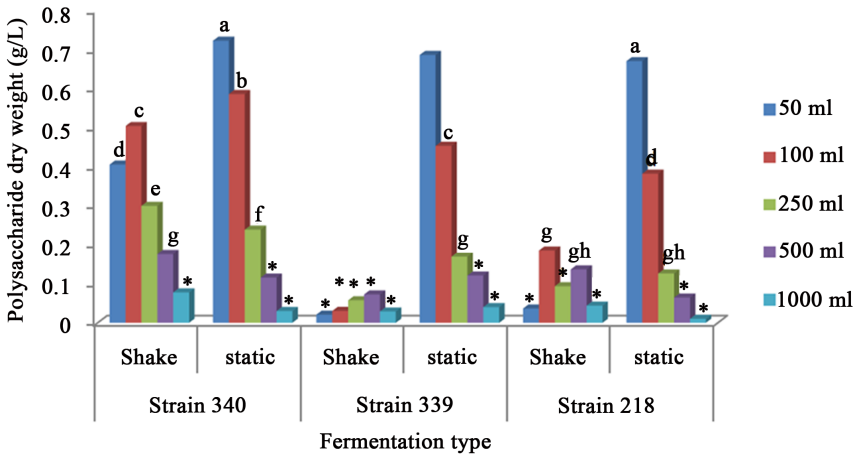

Figure 4. Crude exopolysaccharide in 3 strains of *L. squarrosulus* grown in 5 different volumes of optimized medium (50 - 1000 mL) with or without agitation. Bars with same letter or symbol (*) indicate that the means are not significantly different ($p < 0.0001$).

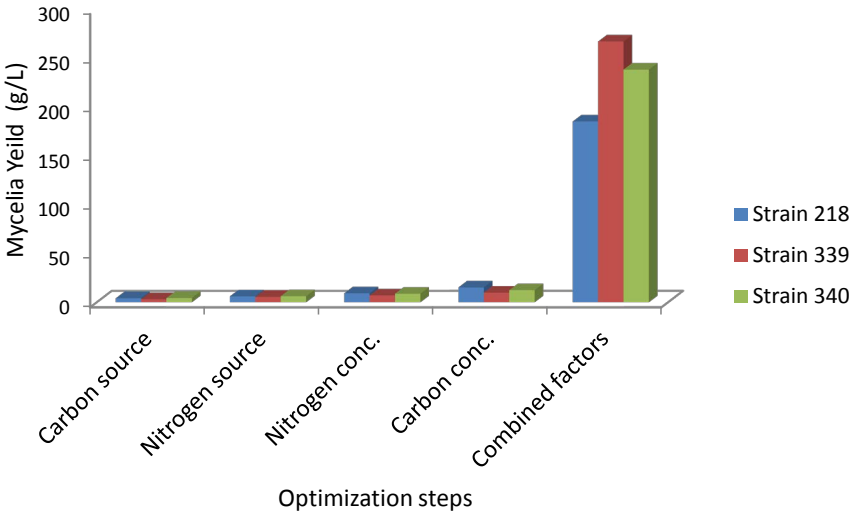

Figure 5. Increase in biomass dry weight at each stage of fermentation optimization process in 3 strains of *L. squarrosulus.* C = carbon; N = nitrogen; conc. = concentration; comb. = combined; scale up was done in IL medium volume.

Since mycelia mass increases with medium volume as discussed above, it is logical that CEPS will decrease as medium volume increases. These results do not agree with Xiao *et al.* [27] who recorded higher CEPS in higher medium than low medium volumes. Higher CEPS was also reported by Ahmad *et al.* [16]. The highest CEPS of 0.72 g/L was observed in 50 mL medium capacity in strain 340 while the lowest (0.01 g/L) was recorded in 1000 mL culture volume in strain 218. Regression analysis shows that 25% of variability in CEPS is due to volume. Since biomass and polysaccharide production are inverse to each other, it was necessary to determine if there is a relationship between them. A regression and correlation analysis shows a weak negative relationship (−0.25) between biomass yield and polysaccharide secretion ($p = 0.002$). This result is in line with earlier report of, Diamantopoulou *et al.* [34], who performed statistical analysis regarding correlation between mycelia mass and EPS for five mushroom strains in static and agitated cultures, and indicated a significant negative relationship between mycelia production with EPS synthesis. Isikhuemhen *et al.* [17] recorded high yield of CEPS (up to 5.13 mg/mL) in the same mushroom (*L. squarrosulus*), albeit in Solid State Fermentation on cornstalk substrate. However in this study, CEPS was generally low in all strains, and maxed out at 0.72 mg/mL in strain 340, 0.69 mg/mL in strain 339 and 0.3 mg/mL in strain 218. In future studies, we will consider other

factors that could increase CEPS yield, such as pH and aeration. Each step of the fermentation optimization process was an improvement on the previous step and mycelia mass increased accordingly (**Figure 5**). The highest mycelia mass was achieved when individual optimized factors were combined together in a single experiment. This is the first report on mycelia and CEPS production by *L. squarrosulus* in submerged fermentation under static and shake conditions.

4. Conclusion

The overall goal of the experiment was to optimize fermentation conditions for mycelia mass production and crude exopolysaccharide secretion in *L. squarrosulus*. There are several factors that can affect fermentation, and the factors considered in the present study do not cover all, but the results suggest that they are particularly influential. We conclude that all the factors studied: strain, nutrient, volume, and agitation affect product yield. Moreover, there is interaction between combinations of these factors. The practical result of the study yields information for the fermentation and biotechnology industry, which use mycelia, polysaccharides, and/or their products for food and health promoting benefits, and for the research community interested in bioactive compounds for further research. Optimally, biomass and exopolysaccharides should be produced in culture medium containing 30 g/L of starch as the carbon source, and 25 g/L of yeast extract as the nitrogen source. During fermentation, if mycelia are desired, a higher volume of 1000 mL is recommended, with agitation at 150 rpm. However if exopolysaccharide is the product of interest, lower volumes of 50 - 100 mL should be used with no agitation. Using such medium and physiological conditions, mycelia yield and/or polysaccharide secretion can be scaled up or down based on desired product.

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