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Effects of carbon source and additives on biomass, exopolysaccharide production and morphology of *Pleurotus ostreatus* in submerged cultivation

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ABSTRACT

Aims: To investigate the influence of carbon sources and additives/surfactants on the mycelium growth and exopolysaccharides (EPS) production, including the morphology during submerged cultivation of *Pleurotus ostreatus* in the minimal-medium as the base medium.
Methodology and results: *Pleurotus ostreatus* was cultivated in different types of carbon sources to investigate the effects of carbon sources to mycelium growth and changes of mycelium morphology which directly affects the synthesis of EPS. In addition, additives or surfactants can increase the bioavailability of less soluble substrates in the cultured medium for the mycelium growth and indirectly affects the EPS production. In this study, the cultivation of *P. ostreatus* in the minimal-medium by using glucose as the carbon source with the addition of lecithin at 1% (w/v) gave the highest EPS production 4.53 ± 0.30 g/L, an increase of about 89.53% when compared to the cultivation without the addition of lecithin. Addition of lecithin changes morphology of the pellets outer layer and under microscope showing a dense hyphal network surrounding the pellets with the sizes of micro pellets almost 0.5-1.5 mm which contributed to the increase of EPS production after 14 days cultivation at 26 °C.
Conclusion, significance and impact of study: The choice of the carbon source should not only be for high productivity rate of mycelium growth and EPS production, but a cheaper alternative source should also be considered. In conclusion, high mycelium biomass and EPS production was achieved either by changes of the morphology through the type of carbon source and addition of additives such as lecithin.

Keywords: Pleurotus ostreatus, exopolysaccharide, submerged, morphology, additives, biomass

INTRODUCTION

Pleurotus ostreatus is an oyster mushroom which belongs to basidiomycete phylum family (Owaid *et al.*, 2015). It is one of the most widely cultivated edible mushrooms under green house (Sánchez, 2010). Mushrooms are spore-bearing fruiting body of fungus that have been utilized due to their high protein content, relatively easy to produce and has better biological efficiency as compared to animal protein (Julian *et al.*, 2018). Recently, applications of polysaccharides from *Pleurotus* sp. in both pharmaceutical and medicinal industry are in high demand. *Pleurotus* species are well known for producing β-glucans as a constituent of the cellular wall of the fruiting body or the mycelium that has medical properties (Avni *et al.*, 2017). In this study, the focus is on the production of exopolysaccharide (EPS) by *P. ostreatus* through submerged cultivation. *Pleurotus ostreatus* can produce an exopolysaccharide known as pleuran [β-(1,3/1,6)-D-glucan] which is known to have anti-cancer, anti-microbial, anti-hypertensive, anti-nociceptive, immune-stimulation and hypocholesterolaemic/anti-atherogenic properties (Jesenak *et al.*, 2013; Vannucci *et al.*, 2013). Unfortunately, to produce commercially important metabolites, a strategy has to be developed in order to achieve high productivity submerged cultivation of filamentous fungi. A high productivity submerged cultivation depends on the morphology of the mycelium during cultivation which contribute to the viscosity of the fermentation medium that may affect oxygen diffusion and bioactive materials production (Nair *et al.*, 2016). The growth of mycelium and bioactive compounds yield are affected by the mycelial morphology that varies depending on the culturing medium either a free suspended mycelium or as a compact pellet, fermentation condition and the strain used (El-Enshasy *et al.*, 2010;

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Zhang and Cheung (2011). In addition, the yield of mycelium and bioactive compounds also depends on the type of carbon source (Papaspyridi *et al.*, 2010; Shi *et al.*, 2011). It is well known that, *Pleurotus* sp. are able to grow to some extent over a wide range of carbon sources from monosaccharide to complex-polysaccharide like arabinose, galactose, glucose, glucuronic acid, sorbitol, glycerol, lactose, mannose, raffinose, sorbose, starch, sucrose and xylose in the submerged cultivation (Hoa and Wang 2015; Kirsch *et al.*, 2016). As study by Smiderle *et al.* (2012) and El-Dein *et al.* (2004) suggested monosaccharide is the best carbon source for optimal EPS production and it promotes growth cultivation of *P. pulmonarius*.

In addition, the supplementation of additives or surfactants in the cultivation broth may change the growth rate and morphology of *P. ostreatus*, which directly influence the EPS production (Kirsch *et al.*, 2016). Generally, different microbial have different effects towards the supplementation of additives in production medium. It is important for us to determine the best additives to be applied for the submerged cultivation of *P. ostreatus*. In this study, the effects of additives such as vegetables oils, glycerol, lecithin, sodium caseinate, sodium alginate, gum Arabic and surfactants (Tween 20, 80, Triton-X 100) to the mycelium's growth, morphology and EPS production of *P. ostreatus* was investigated. The addition of additive will affect the size and morphology of the pellets either in texture (cottony or floccose) or in form and density (high, regular or low and growth) (El-Batal *et al.*, 2015). It is known that surfactant increases the cell membrane's permeability. Cell membrane is the natural barrier of the extra-cellular substrates that transports and produces secretion outside the cell membrane, thus reducing its permeability will increase the polysaccharide production (Zhang and Cheung, 2011). Therefore, the aim of this study is to investigate the influence of carbon sources and additives/surfactants on the mycelium growth and EPS production, including the morphology of *P. ostreatus* that was submerged cultivated in minimal-medium as the base medium.

MATERIALS AND METHODS

Inoculum preparation

Pleurotus ostreatus was used throughout this study. The strain was obtained from My Organic Mushroom Sdn. Bhd., Johor, Malaysia. *P. ostreatus* was grown initially on PDA medium in petri dish for 14 days at 26 °C. The agar plate was scratched gently using a sterilized loop to produce homogenous *P. ostreatus* cell suspension. The surface mycelia were harvested into sterile distilled water using inoculum loop and the homogenized cell suspension was then used as inoculum for further studies. Five milliliters of the homogenized cell suspension to be used as inoculums throughout this study.

Cultivation in different types of carbon source

A minimal-medium modified from El-Enshasy *et al.* (2010) was used as a base medium which consists of 60.0 g/L glucose, 2.0 g/L yeast extract, 0.46 g/L K_2HPO_4, 1.0 g/L $MgSO_4 \cdot 7H_2O$. Prior to sterilization, the initial pH of media was adjusted to pH 5.5. Different types of carbon sources (glucose, sucrose, molasses, lactose, corn starch, mannitol, rice husk and soluble starch) were sterilized separately before being mixed with the other components of the medium during inoculation process to avoid chemical reactions during sterilization. The additive, lecithin, 1% (w/v) was added initially in the cultivation medium and 5 mL of the homogenized cell suspension was transferred in the cultivation flasks. All of inoculated flasks were incubated on an open rotary shaker (Innova 4080, New Brunswick, NJ, USA) at 200 rpm and 26 °C for 14 days.

Supplementation different types of additive/surfactant in the cultured medium

The followings were the additives screened during experimental work: glycerol, palm oil, gum Arabic, gelatin, sodium caseinate, sodium alginate, lecithin and surfactants; Tween 20, Tween 80, Triton X-100. The additives and surfactants in the concentration of 1% (w/v) were added initially in the cultivation medium.

Analysis

Cell biomass measurement

Mycelial dry weights were determined by filtration through pre-weighed Whatman No. 1 filter papers (7.0 cm). Filter papers were oven dried at 65 °C to a constant weight for mycelial dry weight determination.

Extraction of EPS

The cell-free, clear supernatant was used for the EPS determination. The crude EPS was then isolated by 95% ethanol precipitation at the ratio of 1:3. After centrifugation at 6339 ×*g* of 15 min at 4 °C, the EPS pellet was dispersed in aqueous 95% ethanol and centrifuged again (Vamanu, 2012). The final precipitate was dried to a constant weight at 55 °C. The EPS yield was measured.

Mycelia morphology

The main characteristics of mycelia morphology such as texture, pellet shape and density (high, regular or low) as well as growth were identified by visual observation after the complete colonization of the Petri dishes and under bright field microscope with 40× magnification.

Statistical analysis

Experiments were carried out in triplicate and the mean results are presented. Comparison between means was

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carried out using a one-way analysis of variance (ANOVA) and the significant of difference between means was determined by t-test. All statistical analysis was carried out using the statistical package in SPSS 16.0 at 95% least significant difference ($p < 0.05$).

RESULTS AND DISCUSSION

The comparative study on the biomass, EPS production and morphology of *P. ostreatus* with and without addition of lecithin in the submerged cultivation

In this study, submerged cultivation of *P. ostreatus* was cultured in the minimal-medium consisting glucose (60 g/L) as the main carbon source in the shake flask. The mycelium growth and morphology when cultured medium supplemented with and without lecithin 1% w/v were compared. As shown in Table 1, there were no major differences of the biomass in terms of mycelium growth and morphology, which were about 4.74 ± 0.04 g/L and 4.94 ± 0.06 g/L, respectively. As shown in Figure 1, the changes of pellets and growth morphology were observed through visual observation with bright field microscope. The cultivation of *P. ostreatus* without being supplemented with lecithin (Figure 1A) gave a wider distribution of solid pellet between 3-6 mm after 14 days cultivation at 26 °C. Hence, the outer layer of the pellets showed a weak mycelial network as observed under the microscope. However, when cultured in medium supplemented with lecithin at 1% w/v (Figure 1B), the growth was distributed in a mixture of micro pellets (0.5-1.5 mm) with dense mycelial network. Addition of lecithin makes the mycelial morphology varies between pellet and filamentous form. The changes of the outer layer of micro pellets under microscope showed a dense hyphal network surrounding the pellets. As shown in Table 1, the EPS production by addition of lecithin increased up to 1.89 times as compared to without the supplementation of lecithin. In conclusion, from this study the supplement of additives affects the mycelium biomass, EPS production and causes the change of *P. ostreatus* mycelium morphology.

Table 1: The biomass and EPS production in the minimal-medium with and without lecithin 1% (w/v) in the submerged cultivation.

Cultivation in minimal medium	Biomass (g/L)	EPS (g/L)
Lecithin 1% (w/v)	4.74 ± 0.04	4.53 ± 0.30
No lecithin (control)	4.94 ± 0.06	2.39 ± 0.41

Effects of carbon source on the growth, EPS production and morphology of *P. ostreatus* in submerged cultivation

The main aims of this study is to choose the most cost effective carbon source for high EPS production by *P. ostreatus* because carbon source is the main contributor to the cost of cultivation medium. Carbon is an important energy element for the physiological process and biomaterial building blocks for cell synthesis. As shown in Table 2, *P. ostreatus* tends to grow on a wide range of carbon sources either simple sugar or complex sugar. A one-way ANOVA on different treatment effect on the biomass, EPS and yield coefficient was conducted. Molasses was found to significantly affect the biomass than other treatment ($p < 0.05$). For EPS and yield coefficient, glucose was found to be the best treatment. However, glucose was not significantly different from soluble starch and corn starch towards the yield coefficient. From the data, it can be seen that mannitol was the least significant treatment ($p < 0.05$) towards the production of biomass, EPS and yield coefficient. The highest biomass was observed when cultivated in molasses which gave 5.93 ± 0.24 g/L after 14 days cultivation. This is followed by cultivation in sucrose and glucose with biomass production of 4.79 ± 0.02 g/L and 4.68 ± 0.02 g/L, respectively. The lowest biomass production was produced both by corn starch and soluble starch which gave only 2.44 ± 0.09 g/L and 2.39 ± 0.03 g/L, respectively. From the results, it can be concluded that biomass production varied depending on the types of carbohydrate source itself. From the previous studies on fermentation process, glucose was regarded as the best carbon source for *Pleurotus* sp. (Hoa and Wang, 2015). Glucose is a simple sugar that was being preferred by fungi instead of other monomers, this was due to its better substrate catabolism and assimilation during the respiratory pathway process (Wei *et al.*, 2008). The cultivation of *P. ostreatus* in glucose and sucrose (Table 2) can support both mycelium growth and EPS production (Elisashvili, 2012). The yield coefficient of EPS over biomass when glucose and sucrose is the carbon source was 0.96 ± 0.06 g/g and 0.84 ± 0.01 g/g, respectively. However, the growth of *P. ostreatus* in the molasses only gave the highest mycelium growth (5.93 ± 0.24 g/L) but not for EPS production only 3.33 ± 0.18 g/L with the yield coefficient of 0.58 ± 0.03 g/g. From Table 2, even though the biomass of *P. ostreatus* cultivated in corn starch and soluble starch is low, the yield coefficient of EPS is higher with the value of 0.74 ± 0.05 g/g and 0.84 ± 0.01 g/g, respectively.

From Figure 2, the pellet structure depends on the types of carbon sources. The growth of *P. ostreatus* in glucose showed a compact mycelium network without a separate layer of the circular pellets, with a dense short mycelium surrounding the outer layer of pellets which exhibits the highest EPS production (Figure 1). The differences of structure in different types of carbon sources is characterized by 3-4 distinct layers with a compact layer at the center was observed when P. ostreatus was cultivated in sucrose, mannitol and lactose (Figure 2). The inner, less dense layers are clearly subjected to substrate limiting conditions, resulting in autolysis processes within the inner parts of pellet (Prosser and Tough, 1991). However, the pellets morphology for *P. ostreatus* when growing in molasses,

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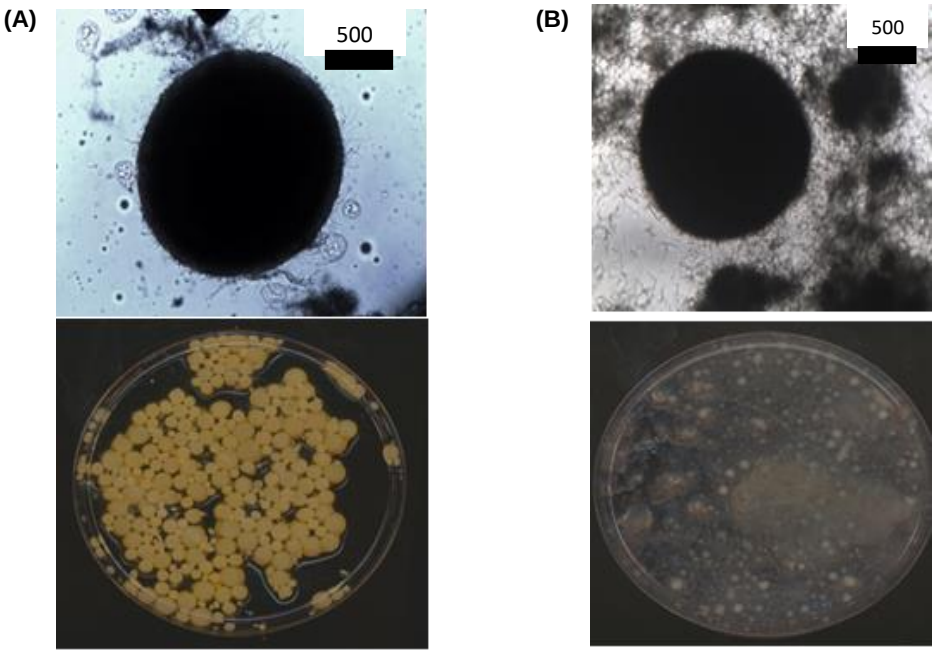

Figure 1: Morphology of *P. ostreatus* under microscope (bar=500 µm) and macro-morphology of *P. ostreatus* in cultured medium with (A) and without (B) supplemented of lecithin 1% (w/v) after 14 days submerged cultivation.

Table 2: The production of biomass, EPS, changes of pH and yield coefficient of EPS by *P. ostreatus* in different types of carbon source supplemented with lecithin 1% (w/v) after 14 days submerged cultivation.

Treatment	Biomass	EPS	Yield coefficient
Glucose	4.685 ± 0.02^{bc}	4.54 ± 0.3^{a}	0.97 ± 0.06^{a}
Sucrose	4.785 ± 0.02^{b}	3.83 ± 0.17^{b}	0.81 ± 0.04^{b}
Lactose	4.2 ± 0.11^{c}	2.08 ± 0.10^{cd}	0.51 ± 0.02^{c}
Mannitol	3.46 ± 0.18^{d}	1.6 ± 0.14^{d}	0.48 ± 0.04^{c}
Molasses	5.93 ± 0.24^{a}	3.33 ± 0.18^{b}	0.58 ± 0.03^{c}
Rice husk	3.45 ± 0.17^{d}	2.58 ± 0.11^{c}	0.78 ± 0.04^{b}
Soluble starch	2.39 ± 0.03^{e}	2.03 ± 0.04^{cd}	$0.84 \pm 0.01a^{b}$
Corn starch	2.44 ± 0.10^{e}	1.86 ± 0.13^{d}	0.75 ± 0.05^{b}

Different letter on the same column are significantly different at $p < 0.05$.

rice husk and corn starch gave 2 distinct layer with a compact layer at the center of pellets with dense hyphae surrounding the pellets. In conclusion, this study found that carbon source also affected the pellets' morphology by changing the density of hyphal networking, as well as to the size and shape of the pellets.

Effects of additive/surfactant on the growth, EPS production and morphology of *P. ostreatus* in submerged cultivation

The influence of additives (sodium caseinate, gum Arabic, sodium alginate, lecithin, glycerol, and palm oil) and surfactants (Tween 20, Tween 80 and Triton X-100) was observed for mycelium growth including effects on the mycelium morphology and EPS production of *P. ostreatus*. In this study, both additives and surfactants gave impact to the mycelial biomass when compared with control cultivation without supplementation of additives. A one-way ANOVA on the different surfactant effect on the biomass, EPS and yield coefficient was conducted. Gum Arabic shows the most significant effect to the biomass than other additive or surfactant ($p < 0.05$). Triton X-100 shows the lowest effect on biomass and its effect was not significant from Tween 20 and Tween 80. There is also a significant effect of the different surfactant on EPS. Lecithin shows the best additive and is significantly affect the production of EPS than another surfactant ($p < 0.05$). For yield coefficient, the best and significant surfactant was also lecithin ($p < 0.05$). In the control cultivation without supplementation of additives, the biomass and EPS production were 4.63 ± 0.11 g/L and 2.26 ± 0.10 g/L (Table 3), respectively. The highest biomass was observed when the minimal-medium was supplemented with gum Arabic (7.64 ± 0.17 g/L) followed by sodium alginate (6.65 ± 0.18 g/L), glycerol (6.24 ± 0.11 g/L), lecithin (5.06 ± 0.09 g/L) and palm oil (3.77 ± 0.06 g/L).

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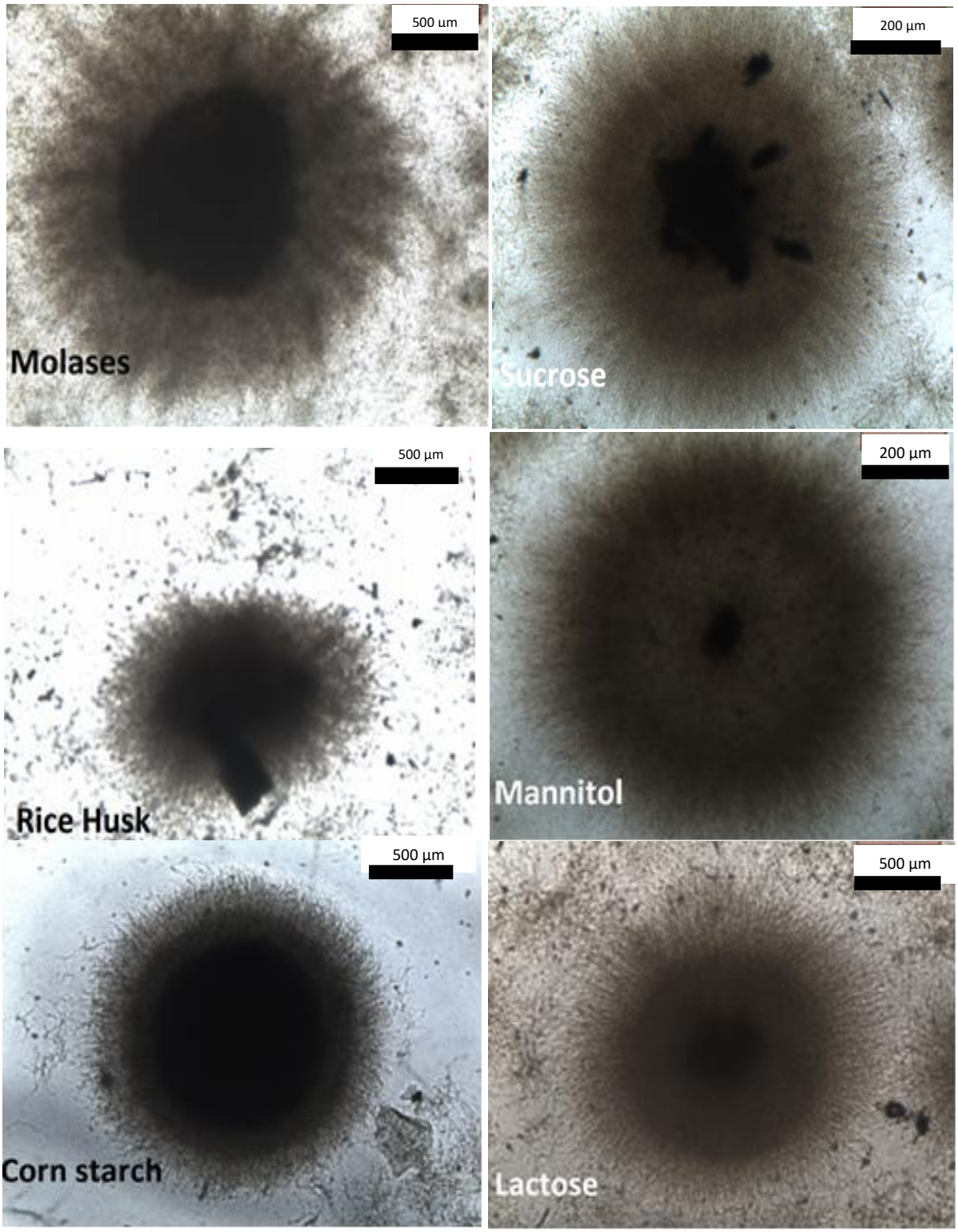

Figure 2: The mycelium morphology under microscope (bar=200-500 µm) of *P. ostreatus* in different type of carbon sources supplemented with lecithin 1% (w/v) after 14 days submerged.

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Table 3: The production of biomass, EPS and yield coefficient of EPS in different types of additives and surfactants after 14 days submerged cultivation of *P. ostreatus*.

Surfactant	Biomass	EPS	Yield coefficient
Control	4.63 ± 0.11^{c}	2.26 ± 0.10^{b}	0.49 ±0.00^{c}
Sodium casinate	2.92 ± 0.08^{e}	0.97 ± 0.06^{de}	0.34 ± 0.00^{de}
Gum Arabic	7.64 ± 0.17^{a}	2.07 ± 0.08^{b}	0.27 ± 0.01^{e}
Sodium alginate	6.65 ± 0.18^{b}	2.25 ± 0.14^{b}	0.34 ± 0.01^{de}
Lecithin	5.06 ± 0.09^{c}	4.68 ± 0.13^{a}	0.93 ± 0.00^{a}
Palm oil	3.78 ± 0.06^{d}	1 ± 0.01d^{e}	0.27 ± 0.00^{e}
Glycerol	6.24 ± 0.11^{b}	1.64 ± 0.07^{c}	0.26 ± 0.01^{e}
Tween 20	2.20 ± 0.02^{fg}	0.91 ± 0.05^{e}	0.42 ± 0.02^{cd}
Tween 80	2.31 ± 0.17^{f}	1.09 ± 0.02^{de}	0.47 ± 0.04^{c}
Triton X-100	1.77 ± 0.06^{g}	1.3 ± 0.13^{cd}	0.74 ± 0.05^{b}

Different letter on the same column are significantly different at $p < 0.05$.

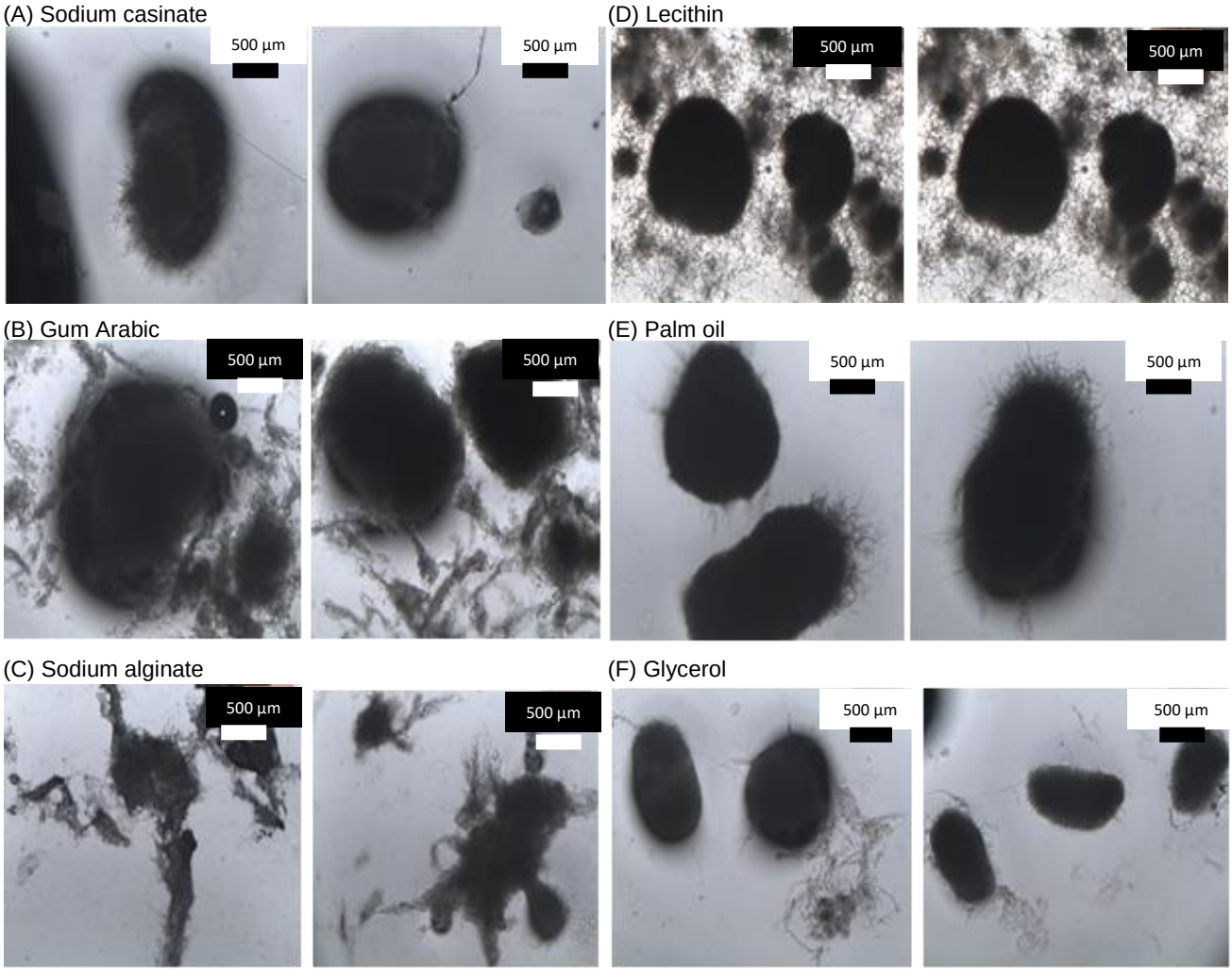

Figure 3: The mycelium morphology under microscope (bar=500 µm) of *P. ostreatus* in different type of additives after 14 days submerged cultivation.

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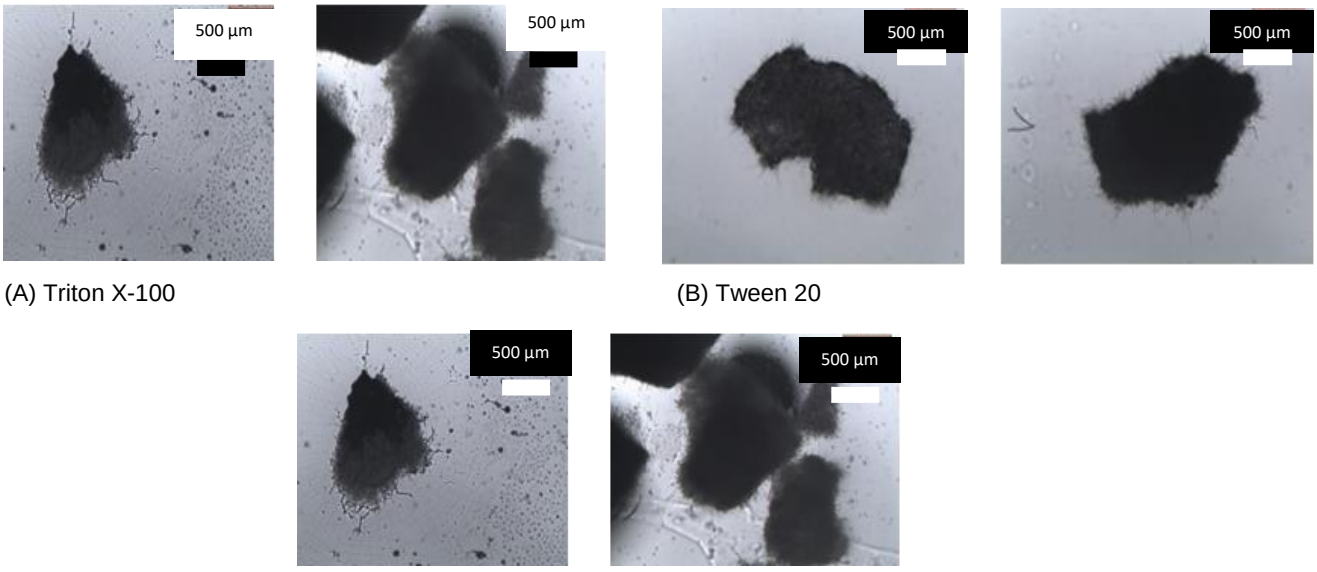

(A) Triton X-100 (B) Tween 20

(C) Tween 80

Figure 4: The mycelium morphology under microscope (bar=500 µm) of *P. ostreatus* in different type of surfactants after 14 days submerged cultivation.

The highest EPS production was obtained when cultured medium supplemented with lecithin followed by supplemented with sodium alginate, gum Arabic, glycerol and palm oil with value of 4.68 ± 0.13 g/L, 2.25 ± 0.14 g/L, 2.07 ± 0.08 g/L, 1.64 ± 0.08 g/L, and 1.0 ± 0.01 g/L (Table 3), respectively. In this study, medium supplemented with lecithin gave the highest yield coefficient value of 0.93 ± 0.01 g/g as compared to control cultivation with only 0.49 ± 0.01 g/g. As a conclusion, gum Arabic is the best surfactant for biomass production while lecithin is the best for EPS production and has the best yield coefficient than other additives.

After 14 days of cultivation, the mycelium morphology of *P. ostreatus* under microscope when supplemented with additives showed solid pellets except for cultivation supplemented with sodium alginate as shown in Figure 3C. The solid pellets between 4-8 mm after 14 days cultivation at 26 °C were observed when supplemented with gum Arabic (Figure 3B) and 3-6 mm of pellets were obtained when the minimal medium was supplemented with lecithin (Figure 3D). Only medium supplemented with gum Arabic and lecithin gave a dense, active hyphal network growth surrounding the pellets. Addition of additives such as gum Arabic, sodium alginate, lecithin and glycerol into the culture medium can serve as carbon sources due to increase of mycelium biomass after 14 days cultivation (Table 3). Only the addition of lecithin can act as stimulators of biosynthesis of EPS including the regulation of cellular membrane permeability since this strategy was proved to be successful in the bacterial fermentation (Elisashvili, 2012). The mycelium growth in the medium supplemented with sodium alginate showed irregulars shape of pellets with the formation of long hyphal branches (Figure 3C).

The cultured medium supplemented with surfactants (Figure 4) did not support the mycelium growth and caused a decrease of the EPS production. Medium supplemented with Triton X-100, Tween 20 and Tween 80 in the minimal-medium (Figure 4A-C), produced solid pellets with a weak mycelial network surrounding the pellets and the mycelium biomass only 1.77 ± 0.06 g/L, 2.20 ± 0.02 g/L and 2.31 ± 0.17 g/L (Table 3), respectively. This is co-currently influencing the EPS production by decreases which were 1.30 ± 0.13 g/L, 0.91 ± 0.05 g/L and 1.09 ± 0.02 g/L (Table 3), with the yield coefficient of EPS 0.74 ± 0.05 g/g, 0.41 ± 0.02 g/g and 0.47 ± 0.04 g/g, respectively. It is known that, surfactants such as Triton X-100, Tween 20 and Tween 80 can increase cell wall permeability and thus might damage the cell membrane or interact with other cellular biomolecules and decreasing the cell vitality (Hsieh *et al.*, 2008). In conclusion, this study demonstrated high mycelium biomass and EPS production was achieved by addition of lecithin which is also proven by Ding *et al.* (2012) in the submerged cultivation of *Ganoderma lucidum*.

CONCLUSION

The changes of mycelium biomass, morphology and EPS production were influenced by the types of carbon source and the supplementation of additives in the submerged cultivation of *P. ostreatus*. Growth of *P. ostreatus* in the minimal-medium with glucose as the main carbon source with addition of lecithin at 1% (w/v) influenced the

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production of mycelium biomass and EPS production via changes in *P. ostreatus* morphology.

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REFERENCES

Avni, S., Ezove, N., Hanani, H., Yadid, I., Karpovsky, M., Hayby, H., Gover, O., Hadar, Y., Schwartz, B. and Danay, O. (2017). Olive mill waste enhances glucan content in the edible mushroom *Pleurotus eryngii*. *International Journal of Molecular Sciences* **18(7), 1564.**

Ding, Z., Wang, Q., Peng, L., Zhang, L., Gu, Z., Shi, G. and Zhang, K. (2012). Relationship between mycelium morphology and extracellular polysaccharide production of medicinal mushroom *Ganoderma lucidum* in submerged culture. *Journal of Medicinal Plants Research* **6(14), 2868-2874.**

El-Batal, A. I., El Kenawy, N. M., Yassin, A. S. and Amin, M. A. (2015). Laccase production by *Pleurotus ostreatus* and its application in synthesis of gold nanoparticles. *Biotechnology Reports* **5, 31-39.**

El-Dein, M. M. N., El-Fallal, A. A., Toson, E. S. A. and Hereher, F. E. (2004). Exoploysaccharides production by *Pleurotus pulmonarius*: Factors affecting formation and their structure. *Journal of Biological Sciences* **7, 1078-1084.**

El-Enshasy, H., Daba, A., El-Demellawy, M., Ibrahim, A., El Sayed and El Badry, I. (2010). Bioprocess development for large scale production of anti-cancer exopolysaccharide by *Pleurotus ostreatus* in submerged culture. *Journal of Applied Science* **10(21), 2523-2529.**

Elisashvili, V. (2012). Submerged cultivation of medicinal mushrooms: Bioprocesses and products. *International Journal of Medicinal Mushrooms* **14(3), 211-239.**

Hoa, H. T. and Wan, C. L. (2015). The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology* **43(1), 14-23.**

Hsieh, C., Wang, H. L., Chen, C. C., Hsu, T. H., Tseng, M. H. (2008). Effect of plant oil and surfactant on the production of mycelial biomass and polysaccharides in submerged culture of *Grifola frondosa*. *Biochemical Engineering Journal* **38, 198-205.**

Jesenak, M., Majtan, J., Rennerova, Z., Kyselovic, J., Banovcin, P. and Hrubisko, M. (2013). Immunomodulatory effect of pleuran (β-glucan from *Pleurotus ostreatus*) in children with recurrent respiratory tract infections. *International Immunopharmacology* **15, 395-399.**

Julian, A. V., Reyes, R. G. and Eguchi, F. (2018). Agro-Industrial Waste Conversion into Medicinal Mushroom Cultivation. *In*: Encyclopedia of Environmental Health, 2nd Edn. Jerome Nriagu (ed.). Elsevier, Oxford, United Kingdom **pp. 1-8.**

Kirsch, L. D. S., Porto de Macedo, A. J., Francisca, M. and Teixeira, S. (2016). Production of mycelial biomass by the Amazonian edible mushroom *Pleurotus albidus*. *Brazilian Journal of Micro*biology **47(3), 658-664.**

Nair, R. B., Lennartsson, P. R. and Taherzadeh, M. J. (2016). Mycelial pellet formation by edible ascomycete filamentous fungi, *Neurospora intermedia*. *AMB Express* **6(31), 1-10.**

Owaid, M. N., Nassar, B. M., Abed, A. M. and Turki, A. M. (2015). Effect of cellulosic matter and container size on cultivation and yield of oyster mushroom *Pleurotus ostreatus*. *Journal of Medicinal Herbs and Ethnomedicine* **1(1), 59-63.**

Papaspyridi, L. M., Katapodis, P., Gonou-Zagou, Z., Kapsanaki-Gotsi, E. and Christakopoulos, P. (2010). Optimization of biomass production with enhanced glucan and dietary fibers content by *Pleurotus ostreatus* ATHUM 4438 under submerged culture. *Biochemical Engineering Journal* **50, 131-138.**

Prosser, J. I. and Tough, A. J. (1991). Growth mechanisms and growth kinetics of filamentous microorganisms. *Critical Reviews in Biotechnology* **10(4), 253-274.**

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology Biotechnology* **85(5), 1321-1337.**

Shi, M., Yang, Y., Li, Y., Wang, Y. and Zhang, Z. (2011). Optimum condition of ecologic feed fermentation by *Pleurotus ostreatus* using soybean curd residue as raw materials. *International Journal of Biology* **3(4), 2-12.**

Smiderle, F. R., Olsen, L. M., Ruthes, A. C., Czelusniak, P. A., Santana-Filho, A. P., Sassaki, G. L., Gorin, P. A. J. and Lacomini, M. (2012). Exopolysaccharides, proteins and lipids in *Pleurotus pulmonarius* submerged culture using different carbon sources. *Carbohydrate Polymers* **87, 368-376.**

Vamanu, V. (2012). Biological activities of the polysaccharides produced in submerged culture of two edible *Pleurotus ostreatus* mushrooms. *Journal of Biomedicine and Biotechnology* **Volume 2012, Article ID 565974.**

Vannucci, L., Krizan, J., Sima, P., Stakheev, D., Caja, F., Rajsiglova, L., Horak, V. and Saieh, M. (2013). Immunostimulatory properties and antitumor activities of glucans. *International of Oncology* **43(2), 357-364.**

Wei, C. H., Zhou, Z., Shi, F. C. and Yong, Q. L. (2008). Optimization for the production of exopolysaccharides from *Fomes fomentariusin* submerged culture and its antitumor effect *in vitro*. *Bioresource Technology* **99, 3187-3194.**

Zhang, B. B. and Cheung, P. C. K. (2011). Use of stimulatory agents to enhance the production of bioactive exopolysaccharide from *Pleurotus tuber-regium* by submerged fermentation. *Journal of Agricultural Food Chemistry* **59(4), 1210-1216.**

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