BIOTROPIA Vol. 16 No. 2, 2009: 96 - 104

EXOPOLYSACCHARIDE PRODUCTION AND ITS BIOACTIVITIES OF THE EDIBLE *PLEUROTUS OSTREATUS* IN SUBMERGED CULTURE

IWAN SASKIAWAN

Research Center for Biology, Indonesian Institute of Sciences
Jl. Raya Bogor Jakarta Km. 46, Cibinong, 16911

ABSTRACT

Submerged cultures were used to study the influence of various carbon sources on the mycelial biomass and exopolysaccharide production of *Pleurotus ostreatus*. The antimicrobial and antioxidant activity of the exopolysaccharide were also determined. The yield of mycelial biomass of 7.06 g/l, 5.12 g/l, 4.66 g/l, and 2.96 g/l was obtained by utilization of maltose, glucose, lactose and starch as a carbon source, respectively. Furthermore, in the medium containing maltose, lactose, glucose, and starch produce 100.05 mg/l, 97.73 mg/l, 78.63 mg/l, and 70.45 mg/l of exopolysaccharide, respectively. The assay of antimicrobial and antioxidant activity showed that the exopolysaccharide is more active to inhibit the growth of *B. subtilis* rather than those of *E. coli* and *S. cerevisiae*. Furthermore, the exopolysaccharide was also indicated to have antioxidant activity.

Key words: *Pleurotus ostreatus*, exopolysaccharide, antimicrobial activity, antioxidant activity

INTRODUCTION

A number of polysaccharides possessing various physiological activities originate from fungi, especially edible and medicinal mushrooms. Many of the edible mushrooms have long been considered to have medicinal value. Mushroom extracts have been reported to have antibacterial, hematological, antiviral, antitumor, hypotensive and hepatoprotective effects (Chang & Miles 1997; Mattila *et al.* 2000). A number of reports on the microbial polysaccharide obtained from higher fungi, such as *Ganoderma lucidum*, *Schizophyllum commune*., *Lentinus edodes*, *Agaricus blazei* are now available (Kim & Kim 1999; Tabata *et al.* 1981; Brauer *et al.* 2002; Mizuno *et al.* 1990).

However, most of the investigators have exerted their efforts to prefer to cultivate these mushrooms on solid artificial media rather than submerged culture. The use of submerged cultures provides a number of potential advantages, for instance a higher mycelial production in a more compact space in a short period of time and lower chances of contamination (Lin & Sung 2006). In addition, exopolysaccharides, which have synergistic effect with mycelia on biological activities, can be simultaneously produced.

The genus *Pleurotus* is a cosmopolitan group of mushrooms with high nutritional value, therapeutic properties and important in various biotechnological and environmental applications. The cultivation of *Pleurotus* sp. is economically important in the food industry worldwide and expanded in the past few years. *P. ostreatus* is the third most important cultivated mushroom for food purposes. A major problem in mushroom cultivation is the lack of information on the cellular processes and the genetic, physiological and environmental controls that lead to the initiation of fruiting body development. Nutritionally, the genus *Pleurotus* has unique flavor and aromatic properties and it is considered to be rich in protein, fiber, carbohydrates, vitamins and minerals. *Pleurotus* sp. are promising medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulation activities (Cohen *et al.* 2002).

The purpose of this study is to optimize the submerged culture conditions to produce mycelial biomass and exopolysaccharide from *P. ostreatus* using several carbon sources. Furthermore, the antibacterial and antioxidant activity of crude exopolysaccharide was also revealed.

MATERIALS AND METHOD

Microorganisms

All of the microorganisms used in this study are the collection of The Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences. A culture of *P. ostreatus* was maintained on a potato dextrose agar (PDA) slant and subcultured every six months. Microorganisms used for the test are the gram-positive bacteria: *Bacillus substilis* and the gram-negative bacteria; *Escheria coli* and yeast *Saccharomyces cerevisiae* and *Candida albicans*. Bacteria were grown on Nutrient Agar (NA) medium (beef extract 3.0 g; bacto peptone 5.0 g; bacto agar 20.0 g on 1l aquadest) and the yeast was grown on Saboroud medium (glucose 20.0 g; bacto peptone 10.0 g; bacto agar 20.0 g on 1l aquadest).

Inoculum preparation and shake flask culture

P. ostreatus grown on PDA medium (dextrose 10.0 g; 1.0 bacto peptone; bacto agar 20.0 g on 1l potato broth) in a petri dish, and then transferred to the seed culture medium by punching out 7mm of the agar plate culture with a sterilized cork borer. The seed culture was grown in a 100 ml flask containing 25 ml of Mushroom Complete Medium (MCM) containing glucose 20 g; KH_2PO_4 0.46 g; K_2HPO_4 1 g; $MgSO_4.7H_2O$ 0.5 g; peptone 2 g; yeast extract 2g on 1l aquadest. The incubation was done at room temperature with shaking of 200 rpm for 4 days. Production of exopolysaccharide and the mycelia growth of *P. ostreatus* were performed in a 250 ml flask containing 50 ml of the MCM inoculated with 5% (v/v) of the seed culture. The culture was then incubated at room temperature with shaking on 200 rpm for several time courses. Various carbon sources (20 g l^{-1}) such as, maltose, lactose and starch were provided instead of glucose as the carbon source in the MCM.

Estimation of mycelial dry weight and analysis of exopolysaccharide

Samples collected at various intervals from shake flask culture were centrifuge at 5.000 rpm for 20 min. and the resulting supernatant was filtered through filter paper and the filtrate was used in the analysis of exopolysaccharide, antimicrobial assay and antioxidant assay. Dry weight of mycelium was measured after repeated washing of the mycelial pellet with distilled water and drying at 70°C for several days to a constant weight. The total sugar content of exopolysaccharide produced by *P. ostreatus* was determined by phenol sulfuric acid method (Dubois *et al.* 1956) using glucose as the standard. Two ml of culture broth in the test tube was added with 0.5 ml of 5% phenol. Then, it was added with 2.5 ml of H_2SO_4, mixed with vortex mixer, let stand for 10 min and then placed in 30°C water bath for 20 min. Read absorbance at 490 nm of the spectrophotometer. The concentration of exopolysaccharide is then determined by measuring the absorbance and comparing to standard curve.

Antimicrobial assay

The antimicrobial activity of exopolysaccharide in the culture broth of *P. ostreatus* was determined by the growth inhibitions of microorganism tested in the medium containing the culture broth of *P. ostreatus*. The microorganism tested *i.e B. substilis* and *E. coli* were grown in liquid NA medium, and of *S. cerevisiae* was grown in liquid Saboroud medium containing culture broth of *P. ostreatus*. The growth parameter of microorganism tested was calculated by measurement of optical density at 600 nm during 0, 2, 4, 6, 8 and 24 hours of incubation time. The growth inhibitions of microorganism tested were expressed in percentage with the equations of:

$$\text{Growth inhibitions (\%)} = \frac{OD_1\, OD_2}{OD_1} \times 100$$

OD1 : OD of microorganism tested in medium without culture broth of *P. ostreatus*
OD2 : OD of microorganism tested in medium with culture broth of *P. ostreatus*

Antioxidant assay

The antioxidant capacity was determined according to the method of Dapkevicius *et al.* 1998 with minor modification. A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.2 mg β-carotene was dissolved in 1 ml of chloroform and 20 µl linoleic acid and 180 µl Tween 80 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 50 ml of distilled water was added with vigorous shaking for 30 min. Five ml of this reaction mixture were placed in a test tube and 5 ml of culture broth of *P. ostreatus* were added and the emulsion was incubated for 150 min at 50°C. The absorbances of the mixtures were measured at 470 nm every 30 min. The same procedure was repeated with synthetic antioxidant BHT (Butylated Hydroxytoluene) as positive control and aquadest as negative control. The antioxidant activity was expressed as the β-carotene bleaching in degradation rate of its absorban using the equation:

$$AA\ (\%) = \frac{Abs_{(0)}\ Abs_{(t)}}{Abs_{(0)}} \times 100$$

$Abs_{(0)}$: Absorbance of the sample at time 0
Abs (t) : Absorbance of the sample at time t, and t = 30 or 60 or 90 or 120 minutes.

RESULTS AND DISCUSSION

Effect of carbon sources on mycelial dry weight and exopolysaccharide production

Mycelial biomass of *P. ostreatus* after 24 days of cultivation is shown in Figure 1. *P. ostreatus* grown in medium containing maltose as carbon sources produced the highest mycelial biomass (7.06 g/l). Utilization of glucose, lactose and starch produced mycelial biomass of 2.96 g/l, 4.66 g/l and 5.12 g/l, respectively. These results are in coincidence with requirements of other mushrooms in submerged cultures. Bae *et al.* (2000) reported a high level of mycelial growth of *Paecilomyces japonica* in media containing maltose in submerged cultures. However, a study by Jonathan and Fasidi (2001) observed that the highest mycelial growth of *Psathyerella atroumbonata* is obtained in medium containing glucose as a carbon source. Thus, the growth curves of *P. ostreatus* with various carbon sources seem to be little difference. Medium containing starch affected in shorter lag phase period of *P. ostreatus* than that of lactose, maltose and glucose. It indicated that starch is suitable for growing of *P. ostreatus* only in the early of its exponential phase.

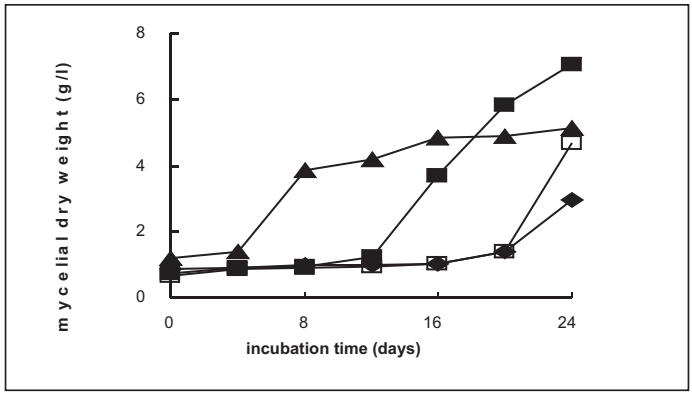

Figure 1. Effect of various carbon sources on mycelial dry weight of P. ostreatus. (♦) Glucose, (■) Maltose, (□) Lactose, and (▲) Starch.

Furthermore, the highest exopolysaccharide production of 100.05 mg/l by *P. ostreatus* was obtained in utilization of maltose as a sugar source (Fig. 2). This result is not in agreement with the results reported by other investigators, who demonstrated that glucose is clearly a good carbon source for exopolysaccharide production in submerged cultures of mushrooms (Xu *et al.* 2003; Nour El-Dein *et al.* 2004; Lin & Chen 2007). However, the profile of exopolysaccharide production by *P. ostreatus* with respect to the carbon source generally was reliable with that of its mycelial biomass. It is interesting to note, because it is often the case with fermentation kinetics of higher fungi that the profile of polysaccharide production was not reliable with that of mycelial growth (Kim *et al.* 2002). In this study it seems that the carbon source can be utilized to improve the production of exopolysaccharide and that mycelial growth determine the high production of exopolysaccharides in *P. ostreatus*. The data about pH during the growth of *P. ostreatus* in submerged medium also showed that the decrease of pH of culture broth with maltose as carbon sources was rather slowly than that of other carbon source (Fig. 3). It is indicated that using maltose as carbon source in the liquid medium is the most suitable for growing of *P. ostreatus*.

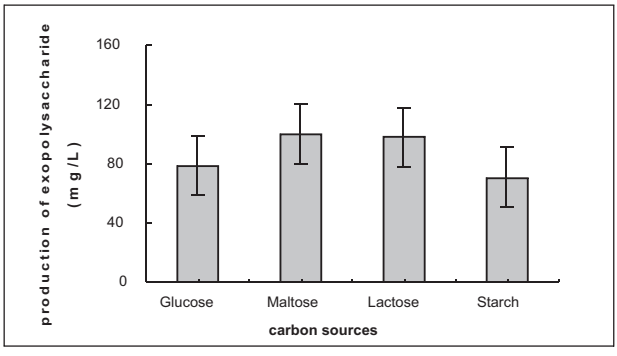

Figure 2. Production of exopolysaccharide by *P. ostreatus* in medium containing various carbon sources.

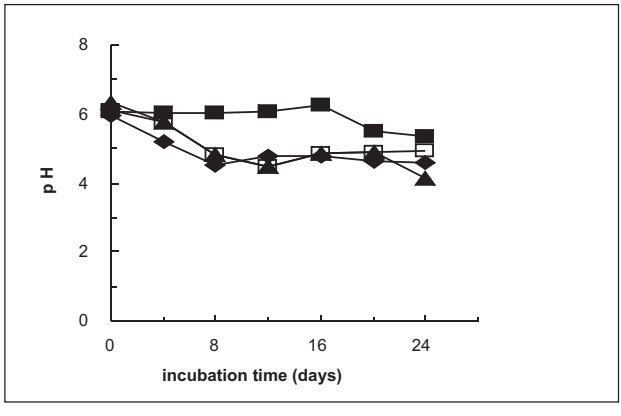

Figure 3. The change of pH on the medium during fermentation process of *P. ostreatus*. (♦) Glucose, (■) Maltose, (□) Lactose, and (▲) Starch.

Antimicrobial activity

The antimicrobial activity of exopolysaccharide in the culture broth of *P. ostreatus* expressed by the growth inhibition of test microorganism is shown in Figure 4. Growth of *P. ostreatus* in medium containing maltose as carbon source exhibited the highest growth inhibition of all test microorganisms. It was 25.33% of *E. coli*, 60.8% of *B. subtilis* and 11.32% of *S. cerevisiae* (Fig. 4). The different sensitivity between gram-positive and gram-negative bacteria could be due to the morphological differences between these microorganisms, gram negative bacteria having an outer polysaccharide membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes. The gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier.

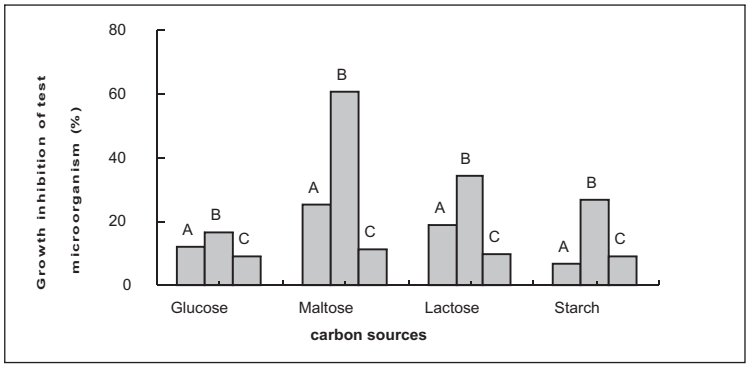

Figure 4. Antimicrobial activity of exopolysaccharide in culture broth of *P. ostreatus* grown in medium containing various carbon sources. (A) *E. coli*, (B) *B. subtilis*, and (C) *S. cerevisiae*.

The growth inhibition of *S. Cerevisiae* against antibacterial polysaccharide of *P. ostreatus* is the lowest among the tested microorganisms grown on various carbon sources. Study by Ramseur *et al.* (2003) showed that the crude extract African mushroom (*P. tuberregium*) sloved using alcohol had antibacterial activity against several food pathogen is microbes, such as *Staphylococcus aureus*, *Salmonella typhimurium* and *E. coli*. Further more, Hirasawa *et al.* (1999) reported that chloroform and water extracted from another Basdiomycetes, *Lentinus edodes* (Berk.) had antibacterial activity against *Streptococcus* sp., *Actinomyces* sp., *Lactobacillus* sp., *Prevotella* sp., and *Porphyromonas* sp.

Antioxidant activity

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperosides arising from linoleic acid oxidation. The degradation rate of β-carotene-linoleic acid system after 120 minutes incubation with polysaccharide of *P. ostreatus* grown on medium using maltose, lactose, glucose and starch as carbon source, aquadest and synthetic

antioxidant, BHT were found to be 1.78%, 8.65%, 9.71%, 27.69%, 27.5%, and 8.14% respectively (Fig. 5). It indicated that polysaccharide of *P. ostreatus* grown on medium containing maltose has higher antioxidant activity than those of on medium containing the other carbon sources. Furthermore, this antioxidant activity of polysaccharide of *P. ostreatus* was also higher than antioxidant of BHT, the synthetic antioxidant. This result is supported the study of antioxidant activity of Basidiomycetes, *Morchella conia* Pers by Turkoglu *et al.* (2006). On the other hand, Jose and Janardhanan (2000) also reported that methanol extract of *P. florida* possesses antioxidant activity.

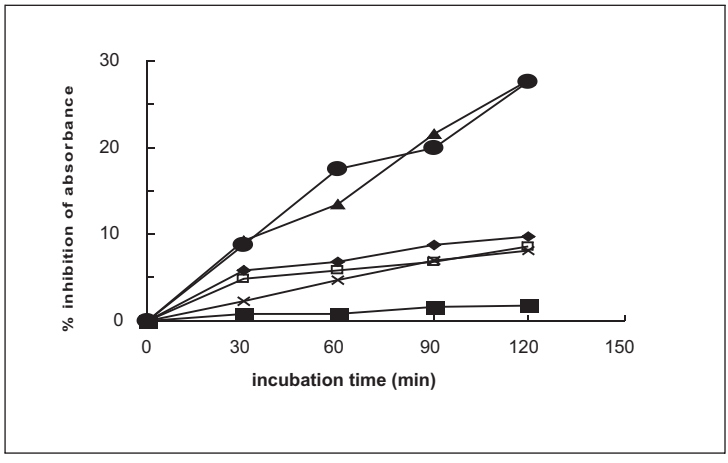

Figure 5. β-carotene bleaching activity of exopolysaccharide in culture broth of *P. ostreatus* grown in medium containing various carbon sources. (●) Aquadest, negative control; (X) BHT, positive control; (♦) Glucose; (■) Maltose; (□) Lactose, and (▲) Starch.

CONCLUSION

The highest production of exopolysaccharide by the edible *P. ostreatus* in submerged culture was obtained while maltose was used as a carbon source compared with the use of glucose, lactose, and starch respectively. This exopolysaccharide is more active to inhibit the growth of *B. subtilis* rather than those of *E. coli* and *S. cerevisiae*. Furthermore, it also indicated to have an antioxidant activity.

ACKNOWLEDGMENT

This research was supported by a research grant from Indonesian Government Funded Projects (DIPA) 2006 of Research Center for Biology, Indonesian Institute of Sciences.

REFERENCES

Bae J.T., Sinha J., Park J.P., Song C.H. and J.W. Yun. 2000. Optimization of submerged culture conditions for exo-biopolymer production by *Paecilimyces japonida*. Journal Microbiology Biotechnology, 10: 482-487.

Brauer D., Kimmons T. and M. Phillips. 2002. Effects of management on the yield and high-molecular-weight-polysaccharide content of shiitake (*Lentinus edodes*) mushrooms. Journal of Agricultural and Food Chemistry, 50: 5333-5337.

Chang S.T. and P.G. Miles. 1997. Mushroom biology. World Scientific Publishing Co. Pte. Ltd. Singapore.

Cohen R., Persky L. and Y. Hadar. 2002. Biotechnological applications and potential of wood-degrading mushrooms of the Genus *Pleurotus*. Applied Microbiology Biotechnology, 58: 582-594.

Dapkevicius A., Venskutonis R., Van Beek T.A. and P.H. Linssen. 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. Journal Science Food and Agriculture, 77: 140-146.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28: 350-356.

Hirasawa M., Shouji N., Neta T., Fukushima K. and Takada K. 1999. Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (Shiitake, an edible mushroom). International Journal of Antimicrobial Agents, 11: 151-157.

Jonathan S.G. and I.O. Fasidi. 2001. Effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata* (Pegler), a Nigerian edible mushroom. Food Chemistry, 22: 479-483.

Jose N and K.K. Janardhanan. 2000. Antioxidant and antitumor activity of *Pleurotus florida*. Current Science, 79: 941-943.

Kim K.C. and I.G. Kim. 1999. *Ganoderma lucidum* extract protects DNA from strand breakage caused by hydroxyl radical and UV irradiation. International Journal of Molecular Medicine, 4(3): 273-277.

Kim S.W., Hwang H.J., Park J.P., Cho Y.J., Song C.H. and J.W. Yun. 2002. Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. Letters in Applied Microbiology, 34: 56-61.

Lin E.S. and S.C. Sung. 2006. Cultivating conditions influence exopolysaccharide production by the edible Basidiomycete *Antrodia cinnamomea* in submerged culture. International Journal of Food Microbiology, 108: 182-187.

Lin E.S. and Y.H. Chen. 2007. Factors affecting mycelial biomass and exopolysaccharide production in submerged cultivation of *Antrodia cinnamomea* using complex media. Bioresource Technology, 98: 2511-2517.

Mattila P., Suonpaa K. and P. Piironen. 2000. Functional properties of edible mushrooms. Nutrition, 16: 694-696.

Mizuno T., Inagaki R., Kanao T., Hagiwara T., Nakamura T., Ito H., Shimura K., Sumiya T. and Asakura A. 1990. Antitumor activity and some properties of water-insoluble heteroglycans from Himematsutake, the fruiting body of *Agaricus blazei* Murill. Agricultural and Biological Chemistry, 54: 2987.

Nour El-Dein M.M, El-Fallal A.A., El-Shahat A.T. and F.E. Hereher. 2004. Exopolysaccharides production by *Pleurotus pulmonarius*: Factors affecting formation and their structures. Pakistan Journal of Biological Science, 7: 1078-1084.

Ramseur R., Goktepe I., Isikhuemhen O. and M. Ahmedna. 2003. Antibacterial activity of African mushroom (*Pleurotus tuberregium*) against select food pathogens. Abstract Association of Research Directors, Inc. 13th Biennial Research Symposium. March 29 April 2, 2003.

Tabata K., Itoh W., Kojima T., Kawabate S. and Misaki K. 1981. Ultrasonic degradation of schizophyllan, an antitumor polysaccharide produced by *Schizophyllum commune*. Carbohydrate Research, 89: 121-135.

Turkoglu A., Kivrak I., Mercan N., Duru M., Gezer K. and H. Turkoglu. 2006. Antioxidant and antimicrobial activities of *Morchella conica* Pers. African Journal of Biotechnology, 11: 1146-1150.

Xu C.P., Kim S.W., Hwang H.J. and Choi J.W. 2003. Optimization of submerged culture conditions for mycelial growth and exo-polymer production by *Paecilomyces tenuipes* C240. Process Biochemistry, 38: 1025-1030.