Effects of Cultivating Conditions on the Water Soluble Polysaccharides Content of *Ganoderma lucidum* Mycelium in Submerged Flask Culture

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Abstract. The carcinostatic substance in *Ganoderma lucidum* (Fr.) Karst (Polyporaceae) is a water soluble polysaccharides (WSP) which might be useful in immunotherapy. Attempt to produce effective substances from cultured mycelia is important to carry out since solid cultivation is a time consuming and quality fluctuating. The effects of cultivating conditions on the water soluble polysaccharides content of *G. Lucidum* mycelium were investigated in submerged flask cultures. Culture from fruiting bodies was maintained on potato dextrose-agar slope. Slopes were inoculated and incubated at 30°C for 7 days, and stored at 4°C. The flask experiments were performed in 100 ml erlenmeyer flasks containing 20 ml of the sterilized media. Actively growing mycelia (1 piece, 5 mm X 5 mm) from a newly prepared slant culture (about 7 days incubation at 30°C) were inoculated into the flask. The pH was measured and adjusted to the desired value by addition of either 4 M HCl or 2.5 M NaOH. Incubation temperature were 20, 25, and 30°C. At the end of inoculation period (14 days) mycelium consisting of individual pellets was harvested and wash for the analysis. WSP content was analysed using phenol-sulfuric acid method. The optimal initial pH for metabolite production would depend on the culture medium. Generally, high values of pH, such as 9, negatively affect both cell growth and WSP production. The optimum temperature range for the high *G. lucidum* mycelium and WSP production were found to be 25 – 30 °C at pH values 5 – 7 in both of media.

1. Introduction

Ganoderma lucidum (Fr.) Karst (Polyporaceae) is a medicinal mushroom which contains water-soluble polysaccharides as a bioactive substance. Water-soluble polysaccharides (WSP) in the *G. lucidum* fruiting bodies has been discovered to be medically active as immunomodulatory, antioxidant, anti-inflammatory, and antitumor. *G. lucidum* polysaccharides have represented a variety of immune modulating effects. Polysaccharides from *G. lucidum* was a promising biological response modifier and immune potentiator [1]. It was found that the polysaccharide extracts from the mycelium of *G. lucidum* had anti-tumor effects against fibro sarcoma in male and female mice and inhibited the metastasis of a tumor to the lung [2]. The fruit bodies of *G. lucidum* grow slowly in nature, their growth is restricted to a specific area. It is necessary to develop an efficient method for the mass production of water-soluble polysaccharides and the mycelial biomass. Some attempts have been made to obtain useful cellular materials or to produce effective by a submerged mycelial culture since conventional cultivation is a

time consuming and quality fluctuating. Submerged culture by fermentation is an alternative approach for an efficient production of higher fungus metabolites. There are several advantages of submerged culture over solid culture for WSP production: high productivity, low costs, availability of convenient control and easy downstream processing [3]. The fundamental information obtained in this work is complementary to those of previous investigations on the submerged culture of *G. lucidum* for the production of bioactive metabolites.

2. Material and methods

2.1 Materials

The fresh mature fruiting bodies of *G. lucidum* were supplied by Gubug Jamur Sitiram from Sleman, Yogyakarta and have been taxonomically verified at Pharmaceutical Biology Division, Faculty of Pharmacy, Universitas Gadjah Mada Yogyakarta. The chemicals and microbiological materials included distilled water, ethanol, sulfuric acid pa, glucose standard, hydrochloric acid, organic potatoes, dextrose bacteriological, agar, and Potato Dextrose Agar, etc.

2.2 Inoculum and media

Inoculum culture from fruiting bodies was maintained on potato dextrose-agar slope. Slopes were inoculated and incubated at 30°C for 7 days, and stored at 4°C. Microbiological media included commercial Potatoes Dextrose Agar and formulated media made from organic potatoes extract, dextrose bacteriological, agar.

2.3 Cultivation of Microorganism

The flask experiments were performed in 100 ml erlenmeyer flasks containing 20 ml of the sterilized media. Actively growing mycelia (1 piece, 5 mm X 5 mm) from a newly prepared slant culture (about 7 days incubation at 30°C) were inoculated into the flask. The pH was measured and adjusted to the desired value by addition of either 4 M HCl or 2.5 M NaOH (pH 5, 7, 9). Incubation temperature are 20, 25, and 30°C. At the end of inoculation period (14 days) mycelium was harvested and wash for the analysis.

2.4 Sample preparation

Mycelia collected from flasks were separated from media, the mycelia was washed twice with distilled water and then weight of dry mycelia was obtained by filtering culture samples through a preweighed filter paper and dried at 80°C to constant weight.

2.5 Water-soluble polysaccharides content

The total water-soluble polysaccharides were determined based on the color reaction of polysaccharides and their derivatives with phenol and concentrated sulfuric acid (Phenol – sulfuric acid method) [4-6]. The total water-soluble polysaccharides were determined as glucose with hydrolyze polysaccharides into glucose monomer. Dried *G. lucidum* mycelia were ground into a powder. The powder of mycelia (0.50 g) was subjected to hot water extraction at 95°C and hydrochloric acid (HCl) 2 M to accelerate the extraction rate. The extract solution was pipetted into a 10-mL centrifuge tube and 1 mL of 5% phenol was added. The mixture was shaken for 2 min and the 5 mL of concentrated sulfuric acid (H_2SO_4) (98% v/v) was added to the solution and shaken for another 5 min. The concentration of water-soluble polysaccharides in the extract solution was determined quantitatively by measuring the absorbance at 490 nm. The standard curve for quantitative analysis of total water-soluble polysaccharides content in the dried *G. lucidum* mycelium was plotted using standard glucose (Sigma, Milwaukee, WI, USA) as a standard solution and distilled water was used as blank solution.

3. Result and discussion

Mycelium weight of *G. lucidum* cultivated in both formulated and commercial media at same pH and temperature had no significant difference. But, WSP production from commercial media was higher than formulated media. It showed that commercial media provide better nutrition than formulated media for WSP production.

Temperature is a very important ecological factor for mycelium growth of fungi. The optimum temperature for the highest yield of *G. lucidum* mycelia in both formulated and commercial medium at incubation pH 5, 7, and 9 was 25 °C. The optimum temperature range for the high *G. lucidum* WSP production were 25 – 30 °C at pH values 5 – 7 in both of media.

Table 1. Dry weight (gram) of mycelial *G. lucidum* in formulated medium at various pH and temperature

Initial pH	Temperature		
	20°C	25°C	30°C
pH 5	2.77 ± 0.06	3.15 ± 0.12	3.11 ± 0.13
pH 7	3.04 ± 0.07	3.31 ± 0.08	2.85 ± 0.09
pH 9	2.89 ± 0.11	2.75 ± 0.06	2.70 ± 0.11

Table 2. Dry weight (gram) of mycelial *G. lucidum* in commercial medium at various pH and temperature

Initial pH	Temperature		
	20°C	25°C	30°C
pH 5	2.92 ± 0.07	3.16 ± 0.09	2.97 ± 0.09
pH 7	3.09 ± 0.15	3.24 ± 0.10	2.89 ± 0.05
pH 9	3.05 ± 0.08	3.11 ± 0.05	2.40 ± 0.06

Table 3. Water-soluble polysaccharides content (%) of mycelial *G. lucidum* in formulated medium at various pH and temperature

Initial pH	Temperature		
	20°C	25°C	30°C
pH 5	38.48 ± 1.15	24.02 ± 1.21	40.44 ± 1.24
pH 7	39.77 ± 1.17	23.03 ± 0.29	36.28 ± 1.09
pH 9	32.62 ± 1.25	25.23 ± 1.11	34.59 ± 1.14

Table 4. Water-soluble polysaccharides content (%) of mycelial *G. lucidum* in commercial medium at various pH and temperature

Initial pH	Temperature		
	20°C	25°C	30°C
pH 5	39.25 ± 1.12	42.46 ± 1.13	45.66 ± 1.20
pH 7	45.46 ± 1.11	53.99 ± 1.14	54.66 ± 1.17
pH 9	40.92 ± 0.97	39.64 ± 1.04	36.03 ± 0.85

The mycelia of various mushrooms species will grow over a wide range of pH values. Nevertheless, for most organisms, the most preferred pH range is from 5 to 7. Lower pH values would be useful to inhibit the growth of bacterial contaminants. pH is also one of the critical factors for controlling mycelial growth and polysaccharide production [8]. Each mushroom has its optimal pH range for development, and it is variable; for example, pH between 4.0 and 7.0 for the mycelium and 3.5 to 5.0 for formation of basidiocarp. The optimum pH for mycelial growth and subsequent fruiting body development is obtained at between 6.5 and 7.0 [9].

The optimum pH for the highest yield of *G. lucidum* mycelia in both formulated and commercial medium at incubation temperature 20 and 25°C was 7. But, the optimum pH for *G. lucidum* growing at incubation temperature 30 °C in both formulated and commercial medium was found to be 5. The optimum pH for the highest *G. lucidum* WSP production in formulated medium was different at each incubation temperature. At incubation temperature 20, 25, and 30°C, the optimum pH was 7, 9, and 5, respectively. However, it is interesting to note that the optimum pH for *G. lucidum* WSP production in commercial medium at various incubation temperature (20, 25, and 30°C) was found to be 7. It demonstrated that optimal initial pH for metabolite production would depend on the culture medium. Generally, high values of pH, such as 9, have negatively affect both cell growth and WSP production.

4. Conclusions

The optimal initial pH for metabolite production would depend on the culture medium. Generally, high values of pH, such as 9, negatively affect both cell growth and WSP production. The optimum temperature range for the high *G. lucidum* mycelia and WSP production were found to be 25 – 30 °C at pH values 5 – 7 in both of media. Commercial media provide better nutrition than formulated media for water soluble polysaccharides production. However, most of commercial media utilized in WSP production are not yet economically available at low cost. In order to meet the requirement of large scale production, further study about media formulation in order to optimize the growth of mycelia of *G. lucidum* and water soluble polysaccharides production in fermenter cultures would be necessary.

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