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The Growth of Mother Cultures and Characteristic of Phytochemical of Pink Oys*ter (Pleurotus flabellatus*) on Different Media

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ABSTRACT

The quality of mother culture is one of Oyster Mushroom cultivation's essential factors as it determines the quality and quantity of the crop. Potato Dextrose Agar (PDA) is the most common media for making the mother cultures of edible mushrooms; therefore, it is essential to find alternative media to increase crop yields. This research aims to find out the most effective alternative media of mother of pink oyster mushroom (*Pleurotus flabellatus*) and the effect on the phytochemical characteristic. The research method used was experimental research, a completely randomized single factor experiment, with four treatments and six repetitions: PDA (*Potato Dextrose Agar*), CDA (*Cron dextrose Agar*), GBDA (*Green bean dextrose Agar*), SDA (*Soy dextrose Agar*). The Agar medium was used to measure pink oyster growth, while broth media were used to determine the biomass weight and the phytochemical compound. The research results showed that the diameter on the CDA medium had the most extensive results among all. Biomass test results showed that the most effective media to obtain biomass were the CDB and GBDB medium. The phytochemical obtained were alkaloids and saponins, while steroids and tannins were not detected in all samples.

Key word: *Biomass, Diameter, Pleurotus flabellatus, Secondary metabolite, Mycelium*

Introduction

Pleurotus flabellatus species, commonly known as Pink Oyster mushrooms, are edible fungi cultivated, especially in developing countries (Khan, et.al., 2013). These mushrooms are healthy food containing high dietary fibre, a medium rate of proteins, amino acids essential, minerals, and vitamins. The protein content varies from 1.6 to 2.5%, and the niacin is about ten times higher than any other vegetable. Moreover, oyster mushrooms are rich in Vitamin C, B complex, and mineral salts required by the human body (Ahmed *et al.,* 2013).

The world market for the mushroom industry was over 10 million tons in 2016 (Bulam *et al.,* 2018). Mushrooms are widely consumed worldwide as a food ingredient meat replacer as it contain high protein, taste good, have a unique texture and halal. It is not surprising that many vegetarians and Muslim consume it as a food substitute for meat (Husain and Huda-Faujan, 2020).

Mushroom cultivation becomes a highly efficient method for recycling agricultural residues and producing nutritious food. Mushroom growth is highly influenced by several factors such as spawn, growing media, pH, temperature, moisture content, and light intensity (Sardar *et al.,* 2015). Just as seed quality is essential to crop production, in mushroom pro-

duction, the quality of spawn is important as it determined the success of mushroom cultivation (Nguyen and Ranamukhaarachchi, 2020).

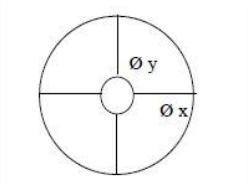
The most often used medium for the growth of the oyster's mother culture in Indonesia is the Potato Dextrose Agar (PDA), which contains a high presentation of carbohydrates, vitamins, and energy that encourage mushroom growth (Griffith *et al.,* 2007). On the other hand, the increasing demand for oyster in the Indonesian market encourages the researcher to find new media sources to improve mother cultures' growth quality. The previous study showed *Pleurotus* could grow in media-rich in protein, carbohydrates, and lipid (Naraian, 2017), such as mung beans (Shi *et al.,* 2016), soybeans (Banaszkiewicz, 2011), and corn kernels (Gehring *et al.,* 2013).

Identifying suitable media, substrate, and incubation time and temperature is essential to obtain high yield and mushroom quality. Previous studies reported the effects of different agar media and substrates on mushroom mycelium's growth and quality. However, most of these involved only a few media in white mushrooms (*Pleurotus osteoporus*). Therefore, the present study aimed to observe the quality of mother cultures and characteristics of phytochemical of pink oyster *(Pleurotus flabellatus*) on different media.

Materials and Methodology

Microorganism and Mother Culture Media

The P. *flabellatus* used in this study was available from the ITB Laboratory. The strain was maintained on potato dextrose agar and sub-cultured every three months. The PDA was made of 200 g of sliced potatoes, approximately 2x2 cm, and boiled with 1000 ml of distilled water in a pan for 15 minutes. Potatoes that had been cooked were filtered, and distilled water was added into the cooking water until it became 1000 ml. Then, the water was boiled again. After that, 20 g of sugar and 20 g of agar were added to the water. The broth was poured into a 100 ml Erlenmeyer flask, then covered with gauze and cotton. This process was also applied to the making of media with alternative materials, which were soybeans, mung beans, and corn kernels which named each, PDA (*Potato Dextrose Agar*), CDA (*Cron dextrose Agar*), GBDA (*Green bean dextrose Agar*), SDA (*Soy dextrose Agar*). The broth was poured into a 100 ml Erlenmeyer flask, then covered with gauze and cotton. This research was conducted with a completely randomized single factor experiment which was repeated six times each.



The PDB was made of 200 g of slices of potatoes approximately 2x2 cm boiled in 1000 ml of distilled water in a pan for about 15 minutes. Potatoes cooked were filtered, and distilled water was added to potato-cooking water until it reached 1000 ml. The water was then boiled again, and 20 g of sugar were added to it. The broth was poured as much as 150 ml into a soy sauce bottle, then covered with gauze and cotton. This process was also applied to the making of media with alternative materials, which were soybeans, mung beans, and corn kernels which named each CDB (*Cron dextrose Broth*), GBDB (*Green bean dextrose Broth*), SDB (*Soy dextrose Broth*)

Incubation and Observation

The incubation process on agar media was conducted in a sterile room. Fungal growth on agar media was observed every day, and the growth was measured. The diameter growth of the pink oyster mushroom mycelia was calculated by measuring the diameter of the radial growth. The calculation formula is as follows:

$$\text{Radial Growth} = \frac{Ø\,x + Ø\,y}{2}$$

Annotation:
Ø x = diameter of x-axis
Ø y = diameter of y-axis

The effect of alternative media on the growth rate of oyster mushroom mycelium was calculated using the following formula in Barnett and Lili V (1951):

$$V = \frac{Wt - W0}{t}\ x\ 100\%$$

Annotation:
V = mycelial growth rate (%)
W0 = initial diameter (cm)
Wt = final diameter (cm)

t = the period of the experiment (day)

Biomass Test

The incubation process on broth media was conducted in a sterile room. The Mycelium of pink oysters were separated from the Potato Dextrose Broth media by filtering it through filter paper after being incubated for 14 days (Ma, Lin *et al.,* 2016). The filtering process used filter paper that had been heated in the oven for 15 hours at 60 °C and had known the dry weight. The pink oyster mushroom mycelia on filter paper were roasted for 24 hours at 60 °C so that the dry weights of the pink oyster mushroom mycelia and filter paper were obtained. Mycelial biomass was calculated using the following formula :

Mycelia biomass = (dry weight of filter paper + dry weight of mycelia) – dry weigh of filter paper

Preparation of Extract

The polyphenol-rich fraction of *Pleurotus flabellatus* was extracted from the Basidiocarps of *Pleurotus flabellatus, dried* and powdered, and kept in ethanol at 25 °C for two days to eliminate triterpenoids, steroids, and other alcohol-soluble compounds. The extract was obtained and filtered with Whatman Number 40. The filtered extract was evaporated with a rotary evaporator at a temperature of 50 °C then oven at 400 °C in order to obtain a crude extract. The extract was then analyzed for a phytochemistry test (Hafsari *et al.,* 2019).

Identification of Phytochemical Compound

Alkaloids Test

This chemical test aimed to detect alkaloids in pink oyster mushrooms. The sample was pink oyster mushroom combined with two drops of H_2SO_4, Wagner's reagent was added into the sample. The presence of alkaloids is characterized by the formation of white deposits by Mayer's reagent, red deposits by Dragendorff's reagent, and brown deposits by Wagner's reagents (Wieczorek *et al.,* 2015).

Saponins Test

This chemical test aimed to detect saponins in pink oyster mushrooms. Mushroom biomass (0.1 g) was combined with a sufficient amount of water and heated for five minutes, then HCL 2 N was added to it. The solution was cooled then shaken. The emergence of foam indicates saponins' presence (Wandati *et al.,* 2013).

Terpenoids and Steroids test

This chemical test aimed to detect steroids in pink oyster mushrooms. Mushroom biomass of 0.1 g was mixed into 2 ml of chloroform, then ten drops of anhydrous acetate and three drops of concentrated H_2SO_4 were added. Positive test result for steroids produces green or blue coloration, and a positive test result for terpenoids produces a red or violet coloration (Patel *et al.,* 2016)

Tannins Test

This test aimed to discover the presence of tannins in pink oyster mushrooms. The $FeCl_3$ 1% were dropped 2-3 times to mushroom extract. The positive result is indicated by bluish-black or green coloration (Ikon *et al.,* 2019).

Results and Discussion

The Mycelium Growth of *P. flabellatus* on Different Agar Media

Based on the results in Figure 1, the diameter colony of pink oysters in each medium showed positive results for mycelia growth. All mycelia were increased from the beginning until day nine. However, the diameter of the colony and the mycelium thickness of pink oyster in each medium was different. This was due to the different nutritional values in each medium that influence mushrooms' growth (Kumara and Edirimanna, 2009).

From Figure 1, it is clear that the highest growth

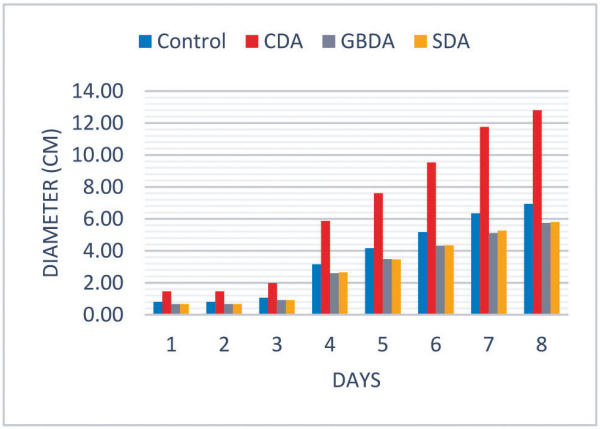


Fig. 1. The Mycelium Growth of *Pleurotus flabellatus* on four different media in nine days observation. PDA (*Potato Dextrose Agar*), CDA (*Cron dextrose Agar*), GBDA (*Green bean dextrose Agar*), SDA (*Soy dextrose Agar*)

of pink oyster was in media CDA on day nine (12,81 cm). It seems that the pink oyster growth depends on the composition carbohydrates, protein, mineral and vitamins due to the corn seeds composition that has a high composition of sugars, protein, crude oil, and variable composition of vitamins and minerals (Nawaz *et al.,* 2018).

The Biomass of *P. flabellatus* on Different Media Broth

Biomass was obtained after the pink oyster mushroom (*Pleurotus flabellatus*) was incubated for 14 days in broth media. The results that have been obtained are presented in Figure 2 below:

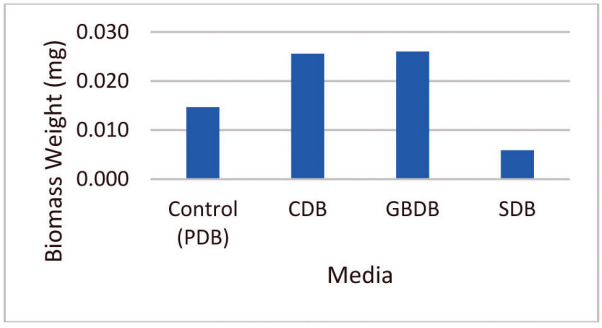


Fig. 2. The Weights of Pink Oyster Mushroom Biomass after 14-Day Culture

Broth medium was effective in producing biomass for CDB and GDBB media it showed with the biomass for 14 days from both media were show the same results. The growth results obtained from the diameters of agar media and the biomass measurements on broth media had differences. This result was similar with Gapiñski *et al.,* (2013), the oyster mushroom mycelia were able to absorb more nutrients on broth media than on agar media because the oyster mushroom mycelium could multiply well on broth media due to the characteristic of nutrition that easy to absorb in broth media and presumably could increase the production of enzymes.

The Phytochemical Characteristic

The result of phytochemical tests is providing in Table 1. The test present of Alkaloid and Saponin while Tannin and Steroid were absent in all experimental treatments. This result indicated that photochemical compound productions might not be affected by the growing media of *P. flabellatus.* The bioactive compounds can be influenced by several factors, such as genetic, physical, and chemical factors.

Alkaloids are heterocyclic compounds that contain nitrogen and recognized for their toxicological relevance (Gargano *et al.,* 2017). The most popular fungal alkaloids are the hallucinogenic indole derivatives, including psilocin (El Enshasy and Hatti-Kaul, 2013) and psilocybin (Jayachandran, Xiao, and Xu, 2017). Saponins are a type of chemical compound that is an amphipathic glycoside, and produced foam when shaken vigorously in solution. The foam is stable and does not come off easily (Hayashi *et al.,* 1997). This result showed that *P.flabellatus* has secondary metabolites which potential as an antitumor, antioxidant and anticholesterol. These compounds can be found in different quantities based on the species of the mushroom, their stage of development, climate conditions, and the availability of solutes nitrogen and phosphorus in the environment (Kovacic *et al.,* 2015).

Conclution

All experiments showed the growth of pink oyster mushroom (*Pleurotus flabellatus*) was good in the CDA medium, which had a growth rate of 86% in 8

Table 1. The Phytochemical test of *Pleurotus flabellatus*, PDA (*Potato Dextrose Broth*), CDA (*Cron dextrose Broth*), GBDA (*Green bean dextrose Broth*), SDA (*Soy dextrose Broth*).

Phytochemical	Phytochemical test on Media and Biomass of Pink Oyster Mushrooms							
	Alkaloid		Saponin		Tannin		Steroid	
	Media	Extract	Media	Extract	Media	Extract	Media	Extract
PDB (control)	+	+++	++	+	-	-	-	-
CDB	+	+++	-	+	-	-	-	-
GBDB	+	+++	-	+	-	-	-	-
SDB	+	+++	-	+	-	-	-	-

Note : (+) Containing (—) No Containing

days, while the control medium had a lower growth rate, 82% in 8 days. The most optimal sugar medium for the growth of pink oyster mushroom (*Pleurotus flabellatus*) was the control medium. The growth rate on pineapple-water medium reached 61% and on sugarcane-juice medium reached 60%. Phytochemical test results show the P. *flabellatus* contained saponins and alkaloids.

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