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PERMAMA

SELECTIVITY TEST FOR SOME OF THE SOLANACEAE FAMILY AS MEDIA FOR THE GROWTH OF OYSTER MUSHROOM (*Pleurotus* sp.) IN VITRO

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Abstract

Oyster mushroom (*Pleurotus* sp.) is a food mushroom and useful for mycoremediation which usually is cultured on PDJ (Potato Dextrose Jelly) in vitro. PDJ is made from potato tubers including one family of *Solanaceae*. However, it is difficult and relatively expensive to acquire and a problem for the majority of mushroom farmers in the lowlands, since potatoes are usually grown in the highlands. This leads to the need to look for alternative selective medium for the growth of oyster mushrooms which is relatively cheap and easy to get among the oyster mushroom growers. This study aims to determine the potential of several members of the family of *Solanaceae* such as tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), and leuncha (*Solanum nigrum*) as selective media for oyster mushrooms media in vitro. The Oyster mushrooms isolated were obtained from Budget P4S district, Rancabungur, Bogor. The research was carried out with pure cultures growing oyster mushrooms on PDJ and alternative media TDJ (Tomato Dextrose Jelly), EDJ (Eggplant Dextrose Jelly), and LDJ (Leuncha Dextrose Jelly). Observation parameters measured were the diameter of mushroom mycelium growth in eight days from a Petri dish in millimeters (mm). The experiments were conducted in a completely randomized design. The treatment given is the type of media used. These experiments were using three treatments which the concentration of plant material were in 150 g/L, 200 g/L and 250 g/L. Results showed that the alternative media TDJ 200 g/L has the largest diameter mushroom mycelium growth than PDJ media and alternative media in the composition of other materials. However, the composition of 150 g/L only alternative media TDJ and EDJ has potential as an alternative selected media for oyster mushrooms than PDJ in vitro.

Keywords: selective media, Solanaceae family, Pleurotus ostreatus, in vitro.

INTRODUCTION

Agribusiness for oyster mushroom is quite promising. Oyster mushrooms can help the healing therapy of diseases such as asthma and cancer. In nature this mushroom grows on wood which experienced weathering or dead, reeds, cane trash, and garbage of sago. If you want to grow oyster mushrooms, then there must be an appropriate fungal culture media.

For some types of fungi which have developed in a special culture medium that allows growth selectively, only certain fungi can grow, thus allowing the rapid identification of fungi (Agrios, 1988). According to the nutritional composition of the constituent, the media can be divided into three categories, which are natural media, semi-synthetic media, and synthetic media. In the semi-synthetic media, in addition to materials, agricultural chemicals used and also a composition can be precisely known. An example of semi-synthetic medium was potato dextrose jelly (PDJ). Media was using one of the plant materials including *Solanaceae*, the potato tubers (*Solanum tuberosum*).

Pure culture mushroom is commonly grown in semi-synthetic media such as potato dextrose jelly (PDJ). The Media is using one of the media available including the *Solanaceae*, the potato (*Solanum tuberosum*). PDJs are often used by researchers and farmers of oyster mushrooms for mushroom growing medium in vitro. However, the media is still deemed too expensive by the farmers. Therefore, we were interested in media selection of plants that are still in one family with potatoes (*Solanaceae* family) such as tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), and leuncha (*Solanum nigrum*) where the material is relatively cheap and easy to obtain. Besides being one family with the potato crop, the plants have a fairly complete nutrition for growing oyster mushrooms such as carbon, nitrogen, vitamins, and minerals. Test selectivity of some *Solanaceae* family media on the growth of the oyster mushroom (*Pleurotus* sp.) is an in vitro study in the search for alternative selective media for pure cultures of oyster mushrooms as a more effective and efficient medium than the PDJ which the material is relatively inexpensive and readily available to be used by researchers and farmers of oyster mushrooms.

MATERIALS AND METHODS

Materials

The materials used in this study are starch, sterile distilled water, sugar, antibiotics, dextrose, jelly, *methylated*, *Pleurotus ostreatus* fungus stock isolates, includes fresh fruit plants in the family *Solanaceae*: potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), and leuncha (*Solanum nigrum*).

The tools used are test tubes, autoclave, microscope, pan, spatula, ladle, stove, bottle, drill, cloth, Bunsen burners, triangular fork, chopsticks, 10 cm diameter Petri dishes, glass objects, scales, blenders, Erlenmeyer flask, gourd peck, gauze, cotton, plastic wrapper, silk, plastic, trays, inoculation loops, isolation/laminar flow, a camera, a pen, and a notebook.

Methods

Isolation of *Pleurotus ostreatus*

Isolation is a process to obtain pure cultures of fungi in the right form for the first time. Therefore, in this process two activities included. First, we want separation of microorganisms from their natural substrate or microorganisms not a destination. Second is the attempt to obtain microorganisms for that purpose in the form of culture. The first is called the isolated culture which isolates. Various isolation methods have been introduced and their use depends on the nature of the fungus lives. Isolation obtained from oyster mushroom of cultivation centers done in Desa Melati P4S Cimulang, District Rancabungur, Bogor. Isolation were then adapted to the temperature of the laboratory for further bred back asexually. Culture that has been obtained is then purified on PDJ that has been sterilized first. Then the cultures were grown for eight days.

Making the media

- **PDJ (*potato dextrose jelly*)**

For the three media recipe, prepare 150 g, 200 g, and 250 g of peeled potatoes then cut into small pieces. Recipes made from potatoes 200 g used as controls. Prepare as much as 1000 mL of distilled water. Cook pieces of potatoes and distilled water for half an hour, then filter to take the extract, then add water to reach a volume of 1000 mL. Add gelatin as much as 15 g and 20 g of dextrose, stirring until it's blended. Insert the mixture into a 250 mL Erlenmeyer flask, then sterilized using an autoclave pressure 15 lb/in^2 at 121°C for 15 minutes. Once autoclaved then air dried the media first so that the temperature is not too hot. Pour about 12 ml of TDJ in each Petri dish and cover with silk fringe cup so that no insects or mites can enter.

- **TDJ (*tomato dextrose jelly*)**

For the three media recipe, prepare 150 g, 200 g, and 250 g of tomatoes that have been peeled. Then cut into small pieces. Prepare each of distilled water, 1,000 ml for each media recipe. Cooked tomatoes pieces and distilled water for half an hour, then filtered to take the extract. Then add water to reach a volume of 1000 mL. For each recipe add the gelatin as much as 15 g and 20 g of dextrose, stir until blended. Insert the mixture into a 250 mL Erlenmeyer flask, then sterilized using an autoclave pressure 15 lb/in^2 at 121°C for 15 minutes.

- **EDJ (*eggplant dextrose jelly*)**

For the three media recipe, prepare 150 g, 200 g, and 250 g eggplant of purple fruit that has been cleaned. Then, cut fruit into small pieces. Prepare each of the 1,000 ml distilled water, for each recipe media. Eggplant pieces and distilled water cooked for half an hour, then filtered to take the extract. Then add water to reach a volume of 1000 mL. For each recipe add the gelatin as much as 15 g and 20 g of dextrose, stir until blended. Insert the mixture into a 250 mL Erlenmeyer flask, then sterile using an autoclave pressure 15 lb/in² at 121°C for 15 minutes.

- **LDJ(*leuncha dextrose jelly*)**

For the three media recipe, prepare 150g, 200 g, and 250 g leuncha fruit that skin has been cleaned. Then cut into small pieces. Prepare each of distilled water, 1,000 ml for each recipe media. Cook Leuncha pieces and distilled water for half an hour, then filtered to take the extract. Then, add water to reach a volume of 1000 mL. For each recipe add the gelatin as much as 15 g and 20 g of dextrose, stir until blended. Insert the mixture into a 250 mL Erlenmeyer flask, then sterilized using an autoclave pressure 15 lb/in² at 121°C for 15 minutes.

In vitro spore germination test

This activity is performed aseptically in isolation. At any media center, in cup inoculated with each fungal isolated from *P. ostreatus*. Pure cultures of fungi were drilled using a small drill to get a slab of mycelium. Inoculation is done by putting a round plate (diameter 7 mm) cultured the fungus was eight days on PDJ, TDJ, EDJ, and LDJ.

Parameter of observations

Data were collected for an average diameter of fungal colonies growing in a Petri dish in units of mm. Response to media fungus is best for mycelia growing on the long and bushy area. The results of further observations are recorded in the journal of observations.

Statistical analysis

Experiments for effectiveness of PDJ, TDJ, EDJ, and LDJ in growing fungus/mushroom *P. ostreatus* in vitro conducted as completely randomized design. The treatment given is the type of media used. These experiments using three treatments, the concentration of plant material 150 g, 200 g, and 250 g per liter medium. The numbers of replications were used three times. Results of experimental data obtained and analyzed by variance (ANOVA) and the median values were compared by Duncan multiple test intervals on the real level of 5%. Statistical analyzes were performed with SAS v.6 program for Windows.

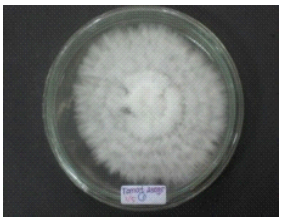
RESULTS AND DISCUSSION

Diameter growth of oyster mushroom mycelium on PDA 250 g/L, TDA 200 g/L, and the TDJ 250 g/L showed significantly different results compared to other media. *Mycelial* growth on PDJ and EDJ are best found on the formulation of 250 g/L (plant material / L). As for the media TDJ and LDJ, the best formulation was 200 g/L. When assessed economically, then the alternative media TDJ most economical value. Since the actual composition of the dose of 150 g/L only media TDJ and EDJ are economically viable for farmers and researchers of oyster mushrooms. This is because farmers typically use a dose of 200 g potato recipe for 1 liter of PDJ. If seen from the carrying capacity of the fungus mycelium growth, TDJ 150 g/L is already supported, which is 1.7 times larger than the PDJ 150 g/L. Meanwhile, the second option is the use of EDJ media.

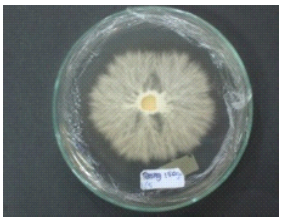
Table 1. The mycelium growth of *P. ostreatus* after 8 days on PDJ, TDJ, EDJ, and LDJ media (mm)

Concentration of plant materials (g/L)	Medium			
	PDJ	TDJ	EDJ	LDJ
150	36.3ed	62.3ab	48.0cd	24.3f
200	63.7ab*	72.0a	40.3ed	42.0de
250	67.3a	67.7a	54.7bc	30.3ef

Data reported are the average of three replications. In each row, means with different letters are significantly different ($p < 0.05$). * = control.



LDA 200 g/L



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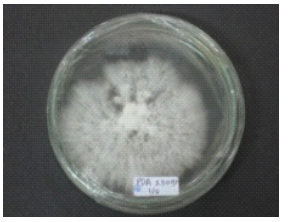
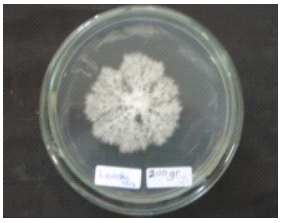


PDA 250 g/L



TDA 200 g/L

Figure 1 Data reported are the average of three replications

The best and the most efficient Solanaceae medium for *P. ostreatus* growth in vitro is TDJ with 200 g/L concentration, it has the better growth than common medium, PDJ with 200 g/L concentration

(control). *Pleurotus* species are recognized for producing β-glucans with important medicinal properties as a constituent of the cellular wall of the fruiting body or of the mycelium (Gern *et al.* 2008). Tomato fruit contains of the best carbon, nitrogen, calcium, vitamin, and minerals which needed for *P. ostreatus* growth. *P. ostreatus* produces laccase (lac) both under conditions of submerged fermentation (SF) and solid-state fermentation (SSF) with all of the carbon and nitrogen sources. In the medium with the best carbon sources, *P. ostreatus* shows the highest Lac activity with $(NH_4)_2SO_4$, as a nitrogen source, with a nitrogen concentration of 20 and 30 mM, respectively (Stajic *et al.* 2006).

For each 1 l medium receipt, 200 g of plant material is the best formulation for TDJ, while EDJ and PDJ are 250 g. TDJ is better not only for *P. ostreatus* mycelium growth, but also economically. TDJ and EDJ are actually better than PDJ at concentration of 150 g/L, thus they can be the alternative media for *P. ostreatus* growth *in vitro.*

Alternative media such as the TDJ and EDJ apparently benefit farmers economically. It is seen from the data that eggplant has cheaper price in the market. Besides, eggplant can grow well in the lowlands around the center of oyster mushroom cultivation. Unlike the relatively stable price of eggplant, tomato price fluctuates (depending on the season), but tomatoes can be grown in the neighborhood where oyster mushroom growers lived. Potato prices in the market are relatively stable, but the price is more expensive than the eggplant, and it is relatively difficult to obtain potatoes around the oyster mushroom growers. This is because potatoes are grown in the highlands, while the majority of the oyster mushroom farmers in the lowlands.

CONCLUSION

The best Solanaceae medium for *P. ostreatus* growth in vitro is TDJ (*Tomato Dextrose Jelly*) with 200 g/l concentration, which better than PDJ as the common medium. TDJ and EDJ(*Eggplant Dextrose Jelly*) can be alternative media for *P. ostreatus* growth in vitro. For each 1 L medium receipt, 200 g of plant material is the best formulation for TDJ, while both EDJ and PDJ are 250 g.

SUGGESTION

The results of in vitro cultured oyster mushrooms need to be tested further in the media such as sorghum in vivo (in a bottle) and baglog media containing bran and sawdust.

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