Sweet Potato and Cassava as Alternative Substrates for Growing Spawn of Shiitake *(Lentinula edodes* B.*)* and Lingzhi *(Ganoderma lucidum* K.*)*

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บทคัดย่อ

ในจำนวนเห็ดที่สามารรถรับประทานได้นั้น เห็ดหอม (*Lentinulaedodes* B.) และเห็ดหลินจือ (*Ganodermalucidum* K.) เป็นเห็ดที่ได้รับความนิยมและสามารถปลูกได้ดีในประเทศอินโดนีเซีย ในปัจจุบันการเพาะเลี้ยงเห็ดในขั้นตอนการเลี้ยงเส้นใยรุ่นแรก (F0) นั้น สามารถใช้อาหารเพาะเลี้ยงได้เพียงแค่อย่างเดียว คือ มันฝรั่ง ในขณะที่ขั้นตอนในการเลี้ยงเส้นใยรุ่นที่สอง (F1) สามารถใช้เมล็ดข้าวโพดได้เพียงอย่างเดียว ซึ่งทั้งมันฝรั่งและเมล็ดข้าวโพดนั้นมีราคาค่อนข้างสูงและค่อนข้างหาได้ยากในอินโดนีเซีย ในงานวิจัยนี้มีเป้าหมายเพื่อที่จะเลือกใช้อาหารเพาะเลี้ยงที่มีราคาไม่แพงและหาได้ง่ายในท้องถิ่น เช่น มันเทศและมันสำปะหลัง เพื่อใช้เพาะเลี้ยงเส้นใยของเห็ดหอมและเห็ดหลินจือ ซึ่งพบว่าการใช้มันเทศ และ มันสำปะหลัง เป็นอาหารเพาะเลี้ยงเส้นใยรุ่นแรกนั้นให้ผลของการเจริญเติบโตของเส้นใยไม่แตกต่างจากการเลี้ยงในอาหารเพาะเลี้ยงที่ทำจากมันฝรั่ง (P>0.05) ในทางกลับกัน พบว่า เมื่อผสมมันเทศและข้าวโพดในอัตรา 60:40 และใช้เป็นอาหารเพาะเลี้ยงเส้นใยรุ่นที่สอง(F1) นั้นให้ผลของการเจริญเติบโตของเส้นใยมากกว่าจากการเลี้ยงในอาหารเพาะเลี้ยงที่ทำจากเมล็ดข้าวโพดเพียงอย่างเดียว (P>0.05) ซึ่งสามารถสรุปได้ว่ามันเทศและมันสำปะหลัง สามารถใช้เป็นอาหารเพาะเลี้ยงสำหรับเส้นใยรุ่นที่หนึ่งและสองของเห็ดหอมและเห็ดหลินจือได้อย่างมีประสิทธิภาพ

คำสำคัญ: เห็ดหอม เห็ดหลินจือ อาหารเพาะเลี้ยง มันเทศ มันสำปะหลัง

Abstract

Edible mushrooms are one of the popular foods of the world. Shiitake (*Lentinula edodes* B.) and Lingzhi (*Ganoderma lucidum* K.) are high-demand medicinal edible mushrooms and widely cultivated in Indonesia. Up till now, potato is the only substrate media used for growing spawn of F0-mycelial growth phase, while corn seeds are the only substrate media used for the F1-mycelial growth phase. Both of the substrate materials are quite costly and their availability is quite rare in some parts of Indonesia. Hence this study proposed an alternative substrate using lower price materials abundantly available in Indonesia, such as sweet potato and cassava for growing spawn of F0-mycelial and F1-mycelial growth phase for Shiitake and Lingzhi. The results showed the utilization of sweet potato and cassava as substrates gave no different result on F0-mycelial growth of both mushroom species compared to potato medium (P>0.05). On the other hand, the mixture of 40% sweet potato and 60% corn seeds as substrate gave a significantly higher F1-mycelial growth rate compared to 100% corn seeds medium (P<0.05). It was concluded that sweet potato and cassava can be used as alternative substrates for F0 and F1-mycelial growth of Shiitake and Lingzhi cultures.

Keywords: Shiitake: Ling Zhi: Sweet potato: Cassava

Introduction

Edible mushroom is important to gourmets for medicine and food supplements [1],[2]. Shiitake (*Lentinula edodes* B.) and Lingzhi (*Ganoderm alucidum* K.) are two species that are widely cultivated in Indonesia and consumed by local people and processed as culinary materials, syrup, and medicinal tea [3]. Since the demand for these mushroom species is high, the research on mushroom tissue culture and cultivation systems is growing rapidly.

The spawn preparation for Shiitake and Lingzhi in Indonesia is still using limited types of substrates. Potato is the only substrate used for spawn preparation for the F0-mycelial growth phase, while the grains, especially corn seeds, are the only substrate used for the F1-mycelial growth phase [4], [5]. Both of the substrate materials are quite costly and their availability is rare in some areas in Indonesia. Therefore, this research aimed to find alternative substrate media that use lower priced materials that freely available in Indonesia. Alternative substrates of interest are sweet potato and cassava for spawn preparation for the F0-mycelial and F1-mycelial growth phases in Shiitake and Lingzhi cultures. The growth-supporting quality of mushroom spawn is determined by the mycelium dry weight at the F0 growth phase, and also the mycelial running rates at the F1 and F2 growth phases.

Material and methods

The experiment was conducted in the laboratory of Microorganisms Biotechnology, Biotechnology Faculty, University of Surabaya, Surabaya, Indonesia in May 2014 to September 2014.

Substrates of F0-mycelial growth preparation

The substrates used for the F0-mycelial growth were potato (PDB), sweet potato (SPDB), and cassava (CDB). The formulation of substrates used is described in Table 1.

Table 1 Substrates formula of F0-mycelial growth media

Substrates	Sweet potato (%)	Cassava (%)	Potato (%)
Control (PDB)	0	0	100
SPDB	100	0	0
CDB	0	100	0
PDB-SPDB	50	0	50
PDB-CBD	0	50	50
SPDB-CBD	50	50	0

Substrates of F1-mycelial growth preparation

The substrates used for the F0-mycelial growth were corn seeds, sweet potato, and cassava. The formulation of substrates is described in Table 2.

Table 2 Substrates formula of F1-mycelial growth media

Substrates	Sweet potato or cassava (%)	Corn seeds (%)
Control	0	100
A	20	80
B	40	60
C	60	40
D	80	20
E	100	0

Spawn preparation of F0-mycelial growth phase (pure culture)

To obtain a pure culture, the tissue culture planting method was used. The Potato Dextrose Broth (PDB) as control was prepared using 250 gr of peeled and sliced potato and 20 gr of dextrose in one liter of water. The composition of the substrates follows the Table 1 formulation. About 50 ml of the formulated medium was poured in each Erlenmeyer flask followed by plugging. The medium was sterilized in an autoclave for 15 minutes at 121^{o}C and kept for a couple of hours for cooling. A small piece of internal tissue of Shiitake and Lingzhi was inoculated aseptically. After 3 days incubation at an ambient temperature, the tissue was covered with a white mycelium on the surface of the medium liquid [6], [7].

Spawn preparation of F1-mycelial growth phase (mother culture)

The mother culture substrates were prepared using boiled corn seeds, sliced boiled sweet potato, and sliced boiled cassava in test bottles following the Table 2 formulation. The test bottles containing the media were sterilized in an autoclave for 15 minutes at 121^{o}C and kept for 24 hours for cooling. A small amount of pure culture was inoculated aseptically and incubated in a dark place at an ambient temperature. The mycelium running of mother culture was observed daily [7], [8].

Spawn preparation of F2-mycelial growth phase

A spatula of mother culture was inoculated aseptically in the F2 medium containing sawdust (no substrate modification) and incubated in a dark place at an ambient temperature. The mycelium running of the mother culture of F2 was observed daily [9], [10] .

Data collection and statistical analysis

The experiment was carried out using Completely Randomized Design (CRD). The data collected consisted of F0-mycelial dry weight with five treatments and a control with five replications, the F1-mycelial running rate with five treatments and a control with five replications, and the F2-mycelial running rate with five replications. The data were analyzed by one way ANOVA test. The treatment means were compared using Tukey simultaneous test.

Results and discussion

Measurement of F0-mycelial dry weight of Shiitake and Lingzhi

The means of the F0-mycelial dry weight ranged from 0.04978 grams to 0.05482 grams for Shiitake and 0.1186 grams to 0.1249 grams for Lingzhi on different substrates as described in Figure 1. The highest F0-mycelial dry weight was observed on PDB, followed by SPDB, SPDB-PDB, CDB, and CDB-PDB, but they were statistically not significant (P>0.05).

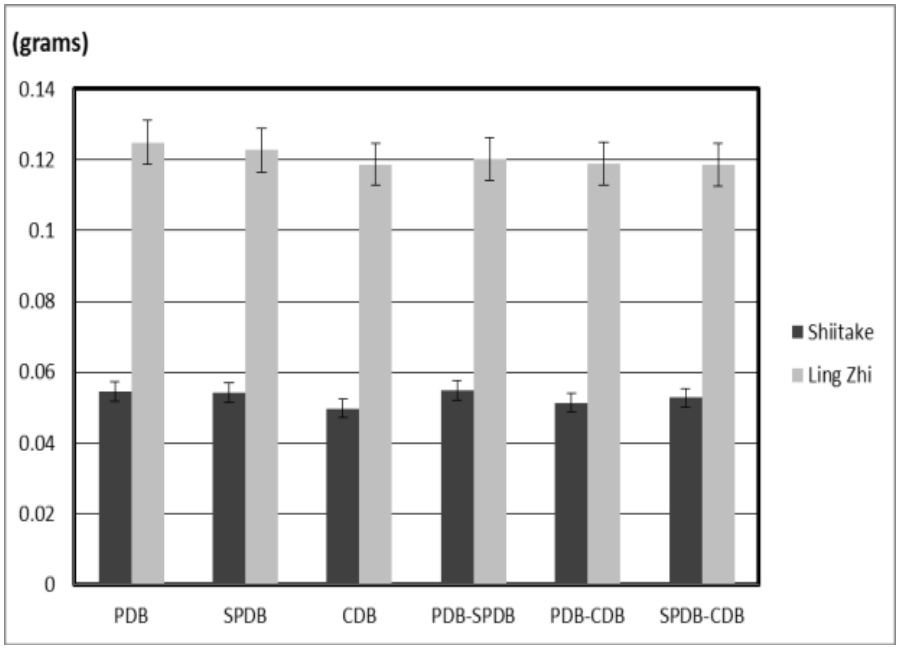

Figure 1 F0-mycelial dry weight of Shiitake and Lingzhi

F1-mycelial running rate of Shiitake and Lingzhi

The means of the F1-mycelial running rate for Shiitake is described in Figure 2 and for Lingzhi in Figure 3. Both of the species have highest the F1-mycelial running rate on the growth substrate containing 60% corn seeds and 40% sweet potato or cassava, which was statistically significant *(p*<0.05).

The F1-mycelial running rate of Shiitake and Lingzhi on the growth substrate containing 40% corn seeds and 60% sweet potato was not statistically different compared to the growth on substrate containing 100% corn seeds (P>0.05). This indicated that corn seeds can be replaced by 60% sweet potato (maximum) as alternative substrate of Shiitake and Lingzhi F1-mycelial growth medium.

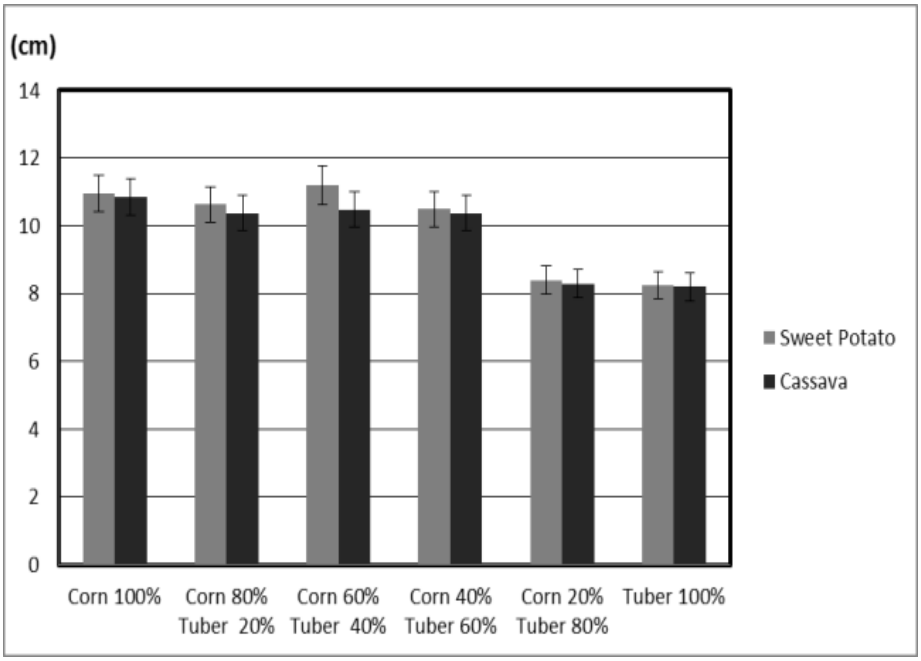

Figure 2 F1-mycelial running rate of Shiitake

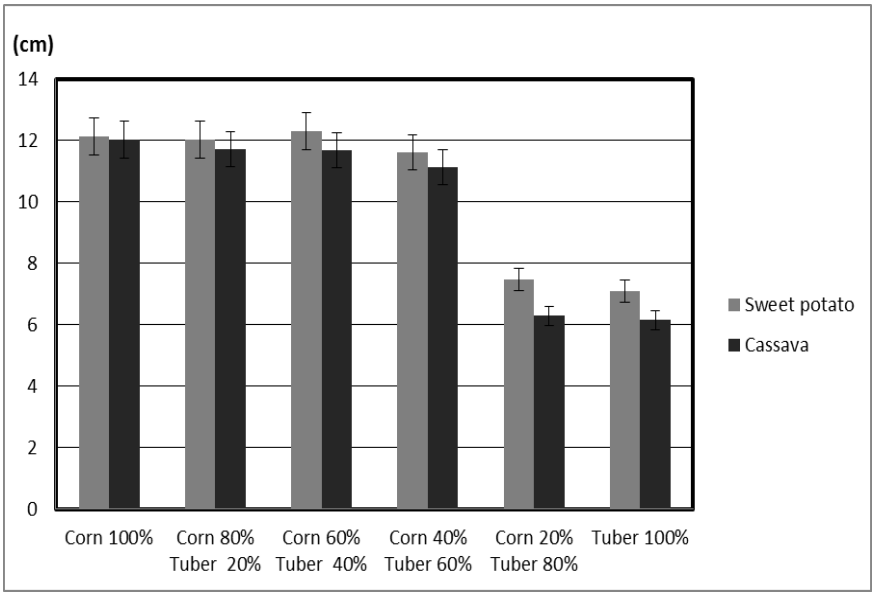

Figure 3 F1-mycelial running rate of Ling Zhi

The F2-mycelial running rate of Shiitake and Lingzhi

The means of the F2-mycelial running rate for Shiitake and Lingzhi is described in Figure 4. Compared to the means of the F2-mycelial running rate from the control substrate (100% corn seeds), the result showed that F2-mycelial growth of Shiitake and Lingzhi from alternative substrates (F1-mycelial grew on 60% corn seeds and 40% tuber) was not significantly different ($P>0.05$). The time required for completion of mycelium running of F2-mycelial Shiitake was 28 days, which is in accordance with the results of a study by Yanamaka [8], whereas F2-mycelial Lingzhi ranged from 25 days to 30 days.

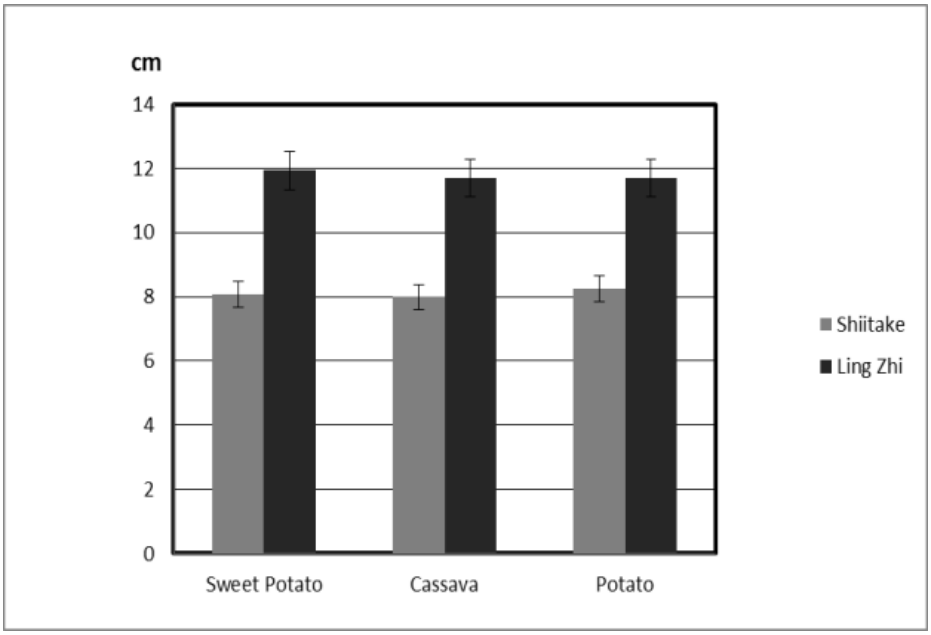

Figure 4 F2-mycelial running rate of Shiitake and Lingzhi

Overall, the results showed that alternative substrates tested in this research can be used as mixture material for the spawn preparation of Shiitake and Lingzhi. Compared to cassava, sweet potato gave the better results for growing spawn for both Shiitake and Lingzhi. It has already been reported that moisture level, pH, and C/N ratio of substrates affect the mycelial growth of cultivated mushrooms [11], [12]. It is possible that sweet potato has suitable properties that support the mycelial growth of Shiitake and Linzhi. Further study needs to be conducted.

Conclusion

The study concluded that sweet potato and cassava can be used as alternative substrates for F0-mycelial growth of Shiitake and

Lingzhi culture, while the mixture of 40% sweet potato and 60% corn seeds as substrate gave a significant result of higher F1-mycelial growth rate of Shiitake and Lingzhi, compared to 100% corn seeds medium ($P<0.05$).

Acknowledgements

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