



# Utilization of MEA medium for isolation and characterization of white oyster mushroom (*Pleurotus ostreatus*) and growth assessment on baglog

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## ABSTRACT

White oyster mushrooms (*Pleurotus ostreatus*) are widely cultivated in Indonesia. Oyster mushroom cultivation is one of the export commodities that help to meet food needs. *Pleurotus* sp. requires an appropriate environment for developing these mushrooms, utilizing suitable planting media. Oyster mushroom media can be straw media or sawdust media. South Sumatra oyster mushroom cultivation uses sawdust media for 57.90% of white oyster mushroom cultivation, and straw media as an alternative for 100% white oyster mushroom cultivation. This research was conducted at the Phytopathology Laboratory, Department of Plant Pests and Diseases, Plant Protection Study Program, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatra. Oyster mushroom observations were conducted in Payakabung Village, Ogan Ilir Regency, Indralaya. The research method used in this study was purposive sampling method which was carried out with 40 glass bottles containing corn groats, bran, and acacia sawdust as oyster mushroom seed media observed for 5 days which were placed in an incubator (20-25 °C) and storage box (26-30 °C), as well as baglog making using sawdust from various types of wood, namely rubber sawdust (Control Treatment) and acacia sawdust (Treatment 2). The development of oyster mushrooms in the first stage is characterized by the formation of fruiting bodies with a length range of 6.5 cm and reaching a diameter range of 15 cm at 40 days. The stalks and hoods of oyster mushrooms are yellowish white. Average growth of oyster mushroom hyphae on MEA media. Observations showed that oyster mushroom isolates on MEA 1 media (fresh oyster mushroom fruiting bodies) had long hyphal growth, and MEA 2 (farmers' seeds) had fast hyphal growth.

**KEYWORDS:** Oyster mushrooms, MEA media, Wood powder

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## INTRODUCTION

The cultivation process of oyster mushrooms consists of four stages: the preparation of pure culture, mother culture, spawn, and production spawn. The pure culture (F0) is obtained from selected fresh oyster mushrooms that are suitable and of good quality for use. A part of the fruiting body of the parent mushroom is taken for cultivation (dan Suparti & Yusron, 2017). The mushroom is then isolated under sterile conditions. The isolation is carried out on Petri dishes containing Malt Extract Agar (MEA) medium. Afterward, the spores germinate into hyphae, which then develop into mycelium (Lestari *et al.*, 2019). The F1 spawn is a derivative of the F0 culture, using grain-based media. The best medium for producing F1 oyster mushroom spawn is corn seed. The F2 spawn (production spawn) is then planted or inoculated into baglogs, which serve

as the growing medium for the fruiting bodies of the oyster mushrooms (Badarina *et al.*, 2023).

The development of white oyster mushrooms (*Pleurotus ostreatus* (Jacq. Fr.) Kumm) has been widely pursued in Indonesia. Oyster mushroom cultivation is one of the export commodities aimed at meeting food demands (Fadlullah & Ma'ruf, 2023). Indonesia has the potential to become a major producer of edible mushrooms due to its tropical climate, which is highly suitable for mushroom cultivation (Ramadhani & Chrismawan, 2020). Edible mushrooms are well-known among local communities for their many benefits, as they are low in calories, carbohydrates, fat, and sodium, and contain no cholesterol. One such edible mushroom that can be cultivated in Indonesia is the oyster mushroom (Widyastuti & Tjokrokusumo, 2022). Oyster mushrooms have high

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economic value. White oyster mushrooms are chlorophyll-free organisms that grow on softwood and obtain nutrients from decomposing organic matter (Canti *et al.*, 2022). The low per capita mushroom consumption in Indonesia (0.18 kg) and the limited productivity per capita remain major challenges and require further development efforts (Machfudi *et al.*, 2021).

The cultivation of oyster mushrooms (*P. ostreatus*) requires an appropriate environment for their development, using a suitable growing medium. The medium commonly used for oyster mushroom cultivation is sawdust. In South Sumatra, sawdust is used in 57.9% of oyster mushroom cultivation, while straw is also used as a 100% substitute material for growing mushrooms (Nunilahwati *et al.*, 2020). The main component of the growing medium is typically rubber wood sawdust. When using straw as a substrate, it must be of good quality and free from pests that may damage the cultivation (Jayanti *et al.*, 2023). The decomposition stage is an important step to reduce pest presence. Decomposition can be carried out by placing the F1 spawn materials or baglogs into sealed polypropylene (PP) plastic bags and allowing them to sit for approximately 5-9 days. During the decomposition process, the temperature inside the PP plastic increases (Susilo *et al.*, 2021).

Indralaya is one of the 16 sub-districts in Ogan Ilir Regency, where the majority of the population has the potential to work as farmers. However, not all farmers are capable of producing and preparing mushrooms. Farmers who are serious about starting an oyster mushroom business must be able to source their spawn to gain greater profits. Considering the number of residents engaged in productive activities, white oyster mushroom cultivation has become a promising alternative for development (Asneti *et al.*, 2015). In oyster mushroom cultivation in Payakabung, North Indralaya Sub-district, mushroom farmers separate specific areas for different stages of the process, such as spawn preparation, sterilization, incubation, and harvesting. The spawn preparation and harvesting are both conducted in a single location known as the *kumbung*. The sterilization and culture areas are kept separate. Sterilization consists of two main activities: sterilizing the baglogs and sterilizing the spawn. During the incubation period, the baglogs are left to rest for 24 hours (Iskandar, 2023).

## MATERIALS AND METHOD

### Study Area

This research was conducted at the Phytopathology Laboratory, Department of Plant Pests and Diseases, Plant Protection Study Program, Faculty of Agriculture, Sriwijaya University Indralaya, South Sumatra. Oyster mushroom observations were carried out in Payakabung Village, Ogan Ilir Regency, Indralaya.

### Study Materials and Tools

The tools used in this study are as follows: Stationery, Autoclave, Cork Drill (0.5 cm), Glass Bottle, Container Box, Bunsen, Petri Dish, Erlenmeyer (500 mL), Ose Needle, Matches, Tweezers,

Ruler, Spatula and Analytical Scales. While the materials used in this study are as follows: Swallow Agar, 90% Alcohol, Aluminum Foil, Aquadest, Bran, Corn Groats, Cotton, Rubber, Instant Malt Extract Agar (MEA), 90% Methanol, PP Plastic, Plastic Wrap, Rubber and seru Wood Powder, and Tissue.

## Research Methods

The sampling method in this study is the purposive sampling method. The source sample of seeds in this study was taken in Payakabung Village, North Indralaya District. The seed research was conducted using 40 clear bottles containing corn grits, bran, and acacia sawdust as oyster mushroom seed media which were observed once every 10 days which were placed in an incubator (20-25 °C) and storage box (26-30 °C), as well as making baglogs using sawdust from various types of wood, namely rubber sawdust as a control treatment (10 replications) and seru sawdust as treatment 2 (10 replications).

## Ways of Working

### Sampling

The collection of oyster mushroom sample seeds from mushroom farmers was carried out at the Payakabung Indralaya location, Ogan Ilir Regency, South Sumatra with good and superior seed characteristics and directly grown on MEA media and purchasing F2 seeds from farmers as a comparison of direct isolation results from oyster mushroom fruit bodies.

### MEA media creation

The media preparation in this study was carried out using Malt Extract Agar (MEA). The MEA media was made using 500 mL of distilled water, 20 g of Malt Extract, and 5 g of agar which were put into a 500 mL Erlenmeyer flask covered with aluminum foil. All tools and materials were wrapped in PP plastic, then autoclaved at a temperature of 121 °C under 1 atm for 5 minutes.

### Selection of oyster mushroom parent seeds

The oyster mushroom parent seeds are taken fresh (fresh), large, and pure white. Seedlings are taken 1 hour before isolation. Seedlings are placed in a Laminar Air Flow (LAF) room so that the oyster mushrooms remain sterile.

### Oyster mushroom isolation (F0)

Isolation in this study was carried out in 2 ways, namely using the oyster mushroom fruit body directly and buying farmer seeds. Isolation using the fruit body by cutting the part between the umbrella and the stem, then the mushroom pieces were washed with running water, and then the outside of the mushroom was sterilized with 70% alcohol. The mushroom pieces were taken with sterile tweezers and cut with a sterile scalpel, take thin pieces of the inside of the tissue or pseudoparenchyma and isolated on a plate using MEA media. Furthermore, incubated

for 3 days until a white cotton-like mycelium mass grew. Then after growing, move or purify and work aseptically. Meanwhile, isolation of oyster mushroom farmer seeds with F1 seed culture, the mushroom seeds were grown on MEA media.

### **Observation of fungal characteristics in dish media**

Observation of oyster mushroom characteristics in the petri dish media in this study was carried out for 14 days. In the observation, the development of the fungus in the Petri dish was observed. After being observed, the color of the mycelium was recorded, and the diameter of its growth was measured and documented.

### **Making baglog**

In this study, the making of baglogs used various types of sawdust such as rubber wood and seru that had been ground. After that, the sawdust was moistened using lime that had been mixed with water and EM4 organic fertilizer which was put into the baglog plastic. Observe how long (cm) the mycelium grew on the baglog.

### **Baglog inoculation**

Inoculation of the baglog in this study was carried out in a sterile room. The top of the Seed bottle that was inoculated on the baglog media was sterilized using Bunsen burner. Take the F1 hyphae using sterile tweezers. Then, the baglog lid was opened, inserting enough mushroom seeds into the baglog, attaching the baglog ring or ring

### **Growth observation in baglog**

In the baglog observation is done once a week. Hyphae that appear on the baglog can be seen on the 30<sup>th</sup> day. Observation of the baglog is measured using a ruler and recorded. In the observation, the difference in hyphae color is seen in each type of sawdust observed. On the 30<sup>th</sup> day, the baglog cover ring is opened.

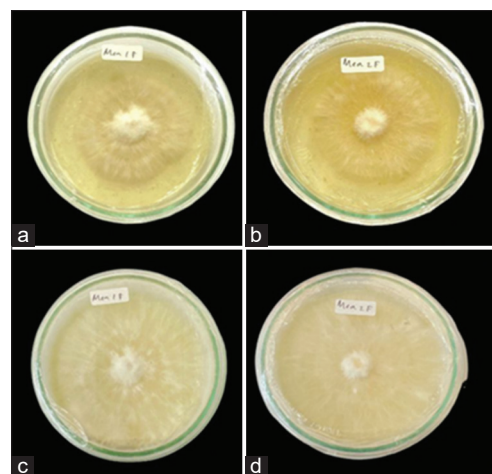
### **Data Analysis**

Data analysis using Excel to determine whether there is a significant difference or not.

## **RESULT AND DISCUSSION**

### **Isolation of F0 Oyster Mushrooms on Dish Media**

The media was made using MEA as the basic material which can be done sterilely. Then isolation with oyster mushroom fruit bodies (F0) was observed for 2 weeks. On the 7<sup>th</sup> day of observation, the growth of the colonies in the cup had begun to spread. On the 14<sup>th</sup> observation, the media in the cup had covered the entire cup and had a yellowish white color. In the isolation of oyster mushrooms, the codes MEA 1 and MEA 2 were used (Figure 1).



**Figure 1:** (a, b) Observations on day 7 (MEA 1 and MEA 2), (c, d) Observations on day 14 (MEA 1 and MEA 2)

### **Characterization of Oyster Mushrooms**

The observation results showed that oyster mushroom isolates on MEA 1 media had slow mycelial growth and MEA 2 had fast mycelial growth. The mycelial growth of MEA media was more significant compared to using PDA media. The average growth of oyster mushroom hyphae on petri dishes using MEA media can be seen in the observation of MEA 1 treatment, Hyphal growth in the first to fourth observations, respectively, namely 1.60 cm, 2.86 cm, 5.44 cm and 6.59 cm. In the observation of MEA 2 treatment, Hyphal growth in the first to fourth observations, respectively, namely 1.19 cm, 2.49 cm, 6.28 cm and 8.80 cm. In the ANOVA test, the results between treatments were not significantly different with the F count ratio being smaller than the F table of 5% of each observation, namely  $3.35 < 4.60$ ;  $1.11 < 4.60$ ;  $0.73 < 4.60$  and significantly different from the F count ratio which is greater than the F table of 5%, namely  $5.81 > 4.60$  (Table 1).

### **Oyster Mushroom Seed Growth**

The average growth of oyster mushroom hyphae on seed media can be seen in the observation of treatment C MEA, Hyphae growth in the first to fifth observations, respectively, were 6.35 cm, 7.89 cm, 8.79 cm, 9.36 cm and 9.82 cm. In the observation of treatment D MEA, Hyphae growth in the first to fifth observations, respectively, were 6.55 cm, 7.79 cm, 8.77 cm, 9.32 cm and 9.77 cm. In the ANOVA test, the results between treatments were not significantly different with the F count ratio being smaller than the F table of 5% of each observation, namely  $0.17 < 4.41$ ;  $0.05 < 4.41$ ;  $0.01 < 4.41$ ;  $0.04 < 4.41$  and  $0.05 < 4.41$  (Table 2).

Based on the results obtained, the growth of the 50<sup>th</sup> day (F1) showed that the seeds stored in the container box (a and b) and the incubator (c and d) both had white mycelium. However, storage in the container box is more susceptible to contamination so that the rate of hyphae growth is inhibited (Figure 2).

Table 1: Mycelium growth on plates on MEA media

Treatments	Mycelium growth in Petri dishes on MEA media for 14 days (cm)			
	1	2	3	4
MEA 1	1.60	2.86	5.44	6.59
MEA 2	1.19	2.49	6.28	8.80
F Hitung	3.35 <sup>tn</sup>	1.11 <sup>tn</sup>	0.73 <sup>tn</sup>	5.81 <sup>tn</sup>
F Tabel 5%	4.60	4.60	4.60	4.60
BNT 5%	0.12	0.18	0.52	0.49

Description: If the F count ratio >F table 5% then the treatment is given the symbol (\*) significantly different and (tn) not significantly different

Table 2: Growth of Oyster Mushroom Seeds

Treatments	Mycelium Growth on Seed Media for 50 Days (cm)				
	1	2	3	4	5
C MEA	6.35	7.89	8.79	9.36	9.82
D MEA	6.55	7.79	8.77	9.32	9.77
F Hitung	0.17 <sup>tn</sup>	0.05 <sup>tn</sup>	0.01 <sup>tn</sup>	0.04 <sup>tn</sup>	0.05 <sup>tn</sup>
F Tabel 5%	4.41	4.41	4.41	4.41	4.41
BNT 5%	0.41	0.36	0.45	0.50	0.55

Description: If the F count ratio >F table 5% then the treatment is given the symbol (\*) significantly different and (tn) not significantly different

### Growth Observation in Baglog

The average growth of oyster mushroom hyphae on the baglog media can be seen in the BKK treatment observation, Hyphae growth in the first to fourth observations, respectively, were 2.95 cm, 10.59 cm, 13.67 cm and 20.62 cm. In the BKS treatment observation, Hyphae growth in the first to fourth observations, respectively, were 2.92 cm, 7.18 cm, 8.62 cm and 10.96 cm. In the ANOVA test, the results between treatments from each observation were not significantly different with a ratio of F Calculation smaller than F table 5%  $0.01 < 4.41$ ; significantly different with a ratio of F Calculation greater than F table 5%, namely  $35.64 > 4.41$ ;  $155.75 > 4.41$ ; and  $120.70 > 4.41$  (Table 3).

Based on the results obtained, the growth of baglogs in weeks 1-4 showed that baglogs made from rubber sawdust (K1-K4) had a slightly dark baglog color while seru sawdust had a reddish brown baglog color (S1-S4). Both grew white mycelium. However, seru sawdust had slowed mycelium growth (Figure 3).

Media treatment gave different results on the growth of isolates (*Pleurotus ostreatus*). The growth rate using MEA (Malt Extract Agar) media showed faster growth compared to using PDA media. The mycelium that grew on MEA media was rather thick and did not form a dense mycelium. MEA media consists of a composition of nitrogen, carbohydrates, sodium, chloride, and agar. Growth is influenced by the nutrient content of the media. The nutritional needs of oyster mushrooms consist of carbon, nitrogen, minerals, and vitamins. Colony growth tends to look like a circular line on the media. The colony continues to grow in an average radius until it reaches the edge of the petri dish (Swandi et al., 2018).

The development of hyphae on the baglog is observed by measuring the growth in diameter. The growth of the baglog

Table 3: Baglog Growth

Treatments	Hyphae Growth on Baglog Media			
	1	2	3	4
BKK	2.95	10.59	13.67	20.62
BKS	2.92	7.18	8.62	10.96
F Hitung	0.01 <sup>tn</sup>	35.64*	155.75*	120.70*
F Tabel 5%	4.41	4.41	4.41	4.41
BNT 5%	0.30	0.47	0.34	0.73

Description: If the F count ratio >F table 5% then the treatment is given the symbol (\*) significantly different and (tn) not significantly different



Figure 2: (a, b) Observation of the 50<sup>th</sup> day of oyster mushroom seedlings placed in a container box with a temperature of (26-30 °C) and (c, d) observation of the 50<sup>th</sup> day of oyster mushroom seedlings placed in an incubator with a temperature of (20-25 °C)

on seru wood is slower than that of rubber wood. Oyster mushrooms can grow on various types of wood that are moist and protected from sunlight. The mycelium formation stage requires a temperature of 22-28 °C, humidity of 80-90% and an oxygen content of 10% (Berutu et al., 2020). White oyster mushrooms on baglogs can grow from soft-textured sawdust and no oil content. Seru wood dust has a hard texture and can inhibit the growth of mycelium on baglogs (Afief et al., 2020). The growth of white oyster mushrooms is closely related to the weathering process in oyster mushrooms and the mushrooms will grow well if the wood has undergone weathering or the weathering process. This is different from rubber sawdust baglogs which have a soft wood texture which is needed for the growth of oyster mushrooms (Herliyana & Muhyi, 2023). Adding EM4 can be a decomposer. EM4 is a mixed culture of beneficial fermentative and synthetic microorganisms consisting of lactic acid, photosynthetic bacteria, actinobacteria, streptomycetes, yeast, and cellulolytic bacteria (Afriadi et al., 2015).

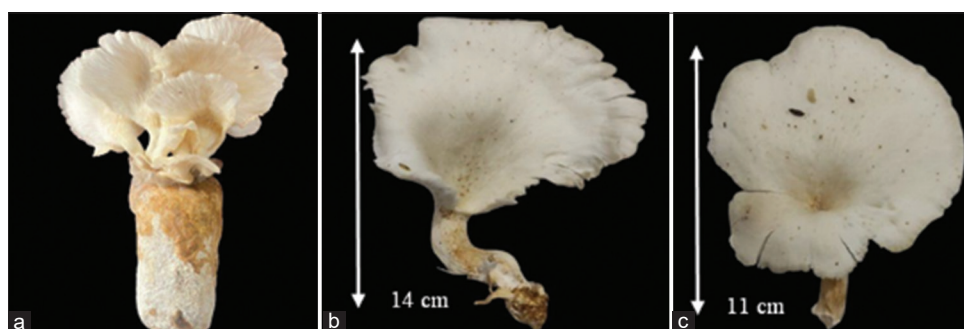
### Morphological Characterization of Oyster Mushroom Fruit Bodies

The morphology of the fruit body obtained in this study has a white color characterization with a large size ranging from  $\pm 14$  cm while the small size is around  $\pm 11$  cm. The mushrooms obtained came from rubber wood baglogs. There are 2 baglogs growing on the baglog (Figure 4).





**Figure 3:** Hyphae growth on rubber baglogs, week 1 (K1), week 2 (K2), week 3 (K3), week 4 (K4), hyphae growth on seru baglogs, week 1 (S1), week 2 (S2), week 3 (S3), and week 4 (S4)



**Figure 4:** (a) Mushroom fruit bodies growing on baglogs, (b) oyster mushrooms growing to a size of 14 cm, and (c) oyster mushrooms growing to a size of 11 cm

The storage temperature of oyster mushroom seeds affects the growth rate or duration of hyphae. At temperatures stored in boxes (26-30 °C) mycelium in seeds requires a longer growth time and is more susceptible to *Trichoderma* sp. Disease in comparison with incubator storage (25 °C) (Saksono & Suprianto, 2019). Mushroom seeds in this study developed well and no seeds were found that did not grow. According to Suryani and Carolina (2017) seed media is one of the important elements for the success and quality of mushrooms and is enriched with grains and contains nutrients that are easily absorbed by mushrooms compared to sawdust media, producing better mushroom seeds. In the study conducted in the manufacture of oyster mushroom seeds, the main raw materials were corn grits, acacia sawdust, and bran. As a comparison, in the manufacture of oyster mushroom seeds, more acacia sawdust is used compared to corn grits, so that it can cause the length of growth of oyster mushroom hyphae in the seeds. Corn grits can be used as a medium for mushroom seeds because of their chemical composition (68%), protein (10%), fat (5%), fiber (2%), and other content (11%).

The constraints identified in this study were related to the stages of making seed media and baglogs, namely the attack of *Trichoderma* sp. This fungus is one of the fungi that can attack oyster mushrooms and can eat their mycelium, thereby killing the mushroom media (baglog). When contaminated fungi grow, control becomes difficult. The infected oyster mushroom

fruit body can cause wrinkled, hollow stems. The emergence of *Trichoderma* sp. is due to the non-sterility in isolating the seeds and baglogs (Mona *et al.*, 2022). *Trichoderma* sp. disease that attacks through the air and on the baglog there are green spots around the baglog (Haryani, 2019).

## CONCLUSION

The results of isolation in pure culture (F0) in MEA (Malt Extract Agar) media have a yellowish white oyster mushroom colony morphology and a colony diameter of 9 cm which is achieved for 14 days. The fruit body grows well on the baglog with the main composition of rubber sawdust, the baglog is full for 50 days.

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