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Functional Group Analysis and Optical Properties of Oyster Mushroom's Mycelium Using Sorghum Media with FTIR Spectrophotometer

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ABSTRACT

The successful establishment of oyster mushroom seeds depends on Potato Dextrose Agar (PDA). In this study, the sterilization level of media varied from level 1 to 3. For each sterilization level, PDA was steamed at 102 ^{o}C for 60 minutes. The best result was observed at the third level, as indicated by mycelial growth. All the spreads (F1) were good and non-tampered with. The result of growing the spread seeds by using sorghum media was not contaminated. The characterization result of FTIR mycelium from pure culture (F0), spread seeds (F1), and planting seeds (F2) indicated the stretching vibration. Stretching vibration showed several functional groups. They were C-O, C-N, C=O, C-H, O-H, and β-D-glukan. These results suggest that the energy required for mycelium growth in the spread seedling culture media is smaller than that required for planting seedling culture media. This follows from the relationship between photon energy and wavenumber, which is directly proportional. The LO values are at wavenumbers 590 cm^{-1} and 905 cm^{-1} for each of the samples F0 and F1. While the TO value is at wave numbers 577 cm^{-1} and 850 cm^{-1} for each sample F0 and F1.

Keywords: *FTIR Spectrophotometer, Longitudinal optic (LO), Potato Dextrose Agar (PDA), Transversal Optic (TO)*

1. INTRODUCTION

The low purchasing power of the Indonesian people has led to reduced consumption of high-protein animal foods. So that the need for vegetable protein becomes an alternative choice to meet the body's protein needs. One alternative to replace high-protein food sources is oyster mushrooms (Pleorotus ostreatus) [1]. The protein content in oyster mushrooms is quite high, ranging from 10.5% to 30.4% per 100 grams of oyster mushroom weight [2]. The protein content in mushrooms is higher than that of other plant foods: mushroom protein has twice the amount of protein as asparagus and potatoes, four times higher than carrots and tomatoes, and six times higher than oranges [3]. In addition to containing protein, oyster mushrooms also contain the minerals K, P, Ca, Na, Mg, and Cu.

The nutritional content of oyster mushrooms provides potential for farmers to start oyster mushroom cultivation businesses. The prospects for developing oyster mushroom cultivation in Indonesia are very promising, as the climate and weather are conducive to oyster mushroom growth. Although the climate and weather are suitable, farmers still face many problems producing high-quality mushroom caps free of contamination. Perseverance and sterile conditions are needed to produce good oyster mushroom seeds, which many farmers find difficult, so many prefer to buy seeds at the market rather than make them themselves. This is an obstacle for small farmers because market-priced seeds are expensive, increasing their costs.

Good oyster mushroom seeds are oyster mushroom seeds produced from pure tissue culture and are free from environmental contamination. Sterilization of Potato Dextrose Agar (PDA) culture media for 60 minutes with three levels of sterilization in this study is expected to minimize contamination in tissue culture media. Sorghum, as a growing medium for both scattered and planted seeds, is used to observe differences in the growth of mushroom seeds compared with other growing media. Previous studies on oyster mushroom production commonly employed corn or wheat grains as the growth medium [4]. However, both materials tend to be more expensive than sorghum, and wheat availability in Indonesia remains limited. Rice can also be used as an alternative, but its small grain size and tendency to clump make it less suitable [5]. Other options, such as legume seeds, are rich in protein, which increases the risk of bacterial contamination. Therefore, sorghum is considered an ideal substrate for oyster mushroom spawn production because it contains complex carbohydrates (approximately 70% starch), protein (8–12%), and essential minerals (Fe, Mg, Zn) that support rapid and dense mycelial growth [6].

FTIR (Fourier Transform Infrared Spectroscopy) characterization plays an important role in oyster mushroom (Pleurotus ostreatus) research, as it enables the detection of key functional groups such as O–H (water, polysaccharides, phenols), C–H (lipids, proteins), C=O (proteins, polysaccharides), N–H (proteins), and C–O–C / C–O–H (polysaccharides such as β-D-glucan and chitin) [7]. FTIR characterization was carried out to see the content of functional groups and molecular bonds in the mycelium of

pure seeds (F0), scattered seeds (F1), and planted seeds (F2). This study aims to observe the effect of sterilization levels in the manufacture of Potato Dextrose Agar (PDA) media, to study the functional groups and optical properties of samples based on the transversal optic (TO) and longitudinal optic (LO) values obtained from the results of the analysis using FTIR.

2. METHODS

The main materials used in this study were white oyster mushrooms, potatoes, agar, dextrose, cloran penicolt, distilled water, sorghum, bran, agricultural lime, and sawdust. The stages of this study include the stages of making white oyster mushroom seeds and FTIR characterization of mycelium molecular bonds.

2.1. Making Oyster Mushroom Seed

The stages of making oyster mushroom seeds begin with preparing Potato Dextrose Agar (PDA) using 200 grams of potatoes, 20 grams of white sugar (dextrose), white agar, phenicolt capsules, and distilled water. Clean potatoes are cut into pieces, boiled in distilled water for 15 minutes, then left to stand until the water turns yellowish. The water is filtered, and distilled water is added to a volume of 1 litre. Dextrose and agar are added to the boiled water until dissolved, then phenicolt capsules are also added to the boiled solution and stirred until evenly distributed. The solution is put into an Erlenmeyer flask and left for 1 hour (sterilization is carried out with 3 levels of variation). PDA is left for 2 days in a sterilization box.

The next stage is isolation with tissue culture. This process begins with preparing healthy, good mushroom fruit bodies and aseptically preparing the mother mushroom. The mushroom fruit body is taken using sterilized tweezers, then planted in a test tube containing media that has been left for two days. All treatments are carried out near a Bunsen burner flame. Incubate the media that has been planted with mushrooms for 4 days. The incubation results are considered successful if white mushroom mycelium grows around the explant and is evenly distributed after two weeks. Pure cultures are ready for use to produce parent seeds.

The next stage is making spread seed., This stage is composed of 1 kg of sorghum, 40 s of dextrose, and 100 g of sawdust. All ingredients are mixed and put into a bottle. Furthermore, the material is sterilized by steaming for 1 hour at a temperature of 100-120 ^{o}C. The next is the inoculation process with mushroom subculture. Inoculation is considered successful if the mushroom seeds grow within 2-3 weeks.

The last stage is planting seeds, which consist of sawdust, bran, corn flour, agricultural lime, dextrose and clean water. Mix all ingredients thoroughly, then gradually add water until the mixture forms a lump when squeezed by hand. When the hand is opened, the mixture should remain compact and not crumble. Cover the mixture with plastic and allow it to decompose (pre-compost) for one day. Fill the substrate mixture into polypropylene baglogs measuring 17 × 25 × 0.3 cm. Sterilize the filled baglogs in an autoclave at 120 °C and 15 psi for 1 hour. Let the sterilized media rest for 24 hours, then inoculate it with the prepared mother spawn.

2.2. Characterization using Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR characterization results provide information on the phenomenon of symmetrical stretching vibration (stretching harmonic vibration) and asymmetrical stretching vibration (stretching anharmonic vibration). These phenomena include vibrational wavenumbers, anharmonic constants, and molecular bond force constants. The characterized mushroom mycelium comes from mycelium in pure culture media (F0), spread seeds (F1), and planted seeds (F2). The FTIR instrument employed was an ABB MB 3000, with data acquisition set within the wavenumber range of 400–4000 cm^{-1}. In addition, FTIR data can be used to study the optical properties of the sauce. These optical properties consist of Longitudinal Optical (LO) and Transversal Optical (TO) values obtained by several processes [8-10]:

The data obtained from FTIR measurements are wavelength and transmittance values. The transmittance value is converted to the reflectance value R using the following equation:

$$A\ (\omega)\ =\ 2\ -\ log[T(\omega)\%] \tag{1}$$

$$R\ (\omega)\ =\ 100\ -\ [T(\omega)\ +\ A(\omega)] \tag{2}$$

Next, the wavelength and reflectance value data are processed using ms. Excel. The reflectance value R is used to determine the refractive index, which consists of the real refractive index (n) and the imaginary refractive index (k), using equations (3) and (4).

$$n(\omega) = \frac{1-R(\omega)}{1+R(\omega)-2cos\varphi(\omega)\sqrt{R(\omega)}} \tag{3}$$

$$k(\omega) = \frac{2sin\varphi(\omega)\sqrt{R(\omega)}}{1+R(\omega)-2cos\varphi(\omega)\sqrt{R(\omega)}} \tag{4}$$

The next step in obtaining the refractive index values n and k requires the value of φ(ω). This value is calculated using the following equation, known as the simplified KK relation (5).

$$\varphi(\omega_j) = -\frac{4\omega_j}{\pi}\Delta\omega\ \sum_i \frac{\ln(\sqrt{R(\omega)})}{\omega_i^2-\omega_j^2} \tag{5}$$

where Δω=ω_(i+1)-ω_i, and if j is an odd number then i = 2, 4, 6, …, j – 1, j+1,… whereas if j is an even number i = 1, 3, 5, …, j – 1, j+1,…Furthermore, from the value of φ(ω) the values of n and k will be obtained.

3. RESULTS AND DISCUSSION

3.1. Potato Dextrose Agar (PDA)

The success of oyster mushroom cultivation depends heavily on the seeds used. To produce pure cultures (F0), good, nutritious, and contamination-free growing media are needed. The media used for the growth of pure cultures is Potato Dextrose Agar (PDA). In this study, variations in sterilisation levels were carried out to produce good PDA Table 1.

media, namely, sterilisation levels 1, 2, and 3. Sterilization level 1, PDA is steamed in a steamer for 60 minutes, and PDA is ready to use. While level 2, PDA is steamed in a steamer for 60 minutes after being left for 24 hours in a sterilization box. Sterilization level 3, PDA that has been steamed for the second time is left again for 24 hours, then steamed again. The measured temperature of each sterilization level is the same, which is at a temperature of 102 ^{o}C. The measured temperature of the three sterilization level treatments is 102 ^{o}C as shown in

Table 1 Sterilization Level Treatment of PDA

Boiling time of potatoes	Sterilization level			Temperature (^{o}C)
	Level 1	Level 2	Level 3	
15 minutes	60	60	60	102
	-	60	60	102
	-	-	60	102

3.2. Pure Breed (F0)

Pure tissue culture from fresh white mushroom fruit bodies was planted on PDA media that had been successfully formed. Pure tissue culture was performed using the same treatment for all PDAs. Table 2 shows that oyster mushroom tissue culture was not all successful. Five repetitions have been carried out for each level of sterilization. Sterilization level 3 successfully produced good white oyster mushroom mycelium, as indicated by the presence of mycelium threads that filled the test tube. Sterilization level 3 successfully produced the best pure culture because the PDA was sterilized three times (3 levels) for 60 minutes which killed the microbes in the PDA due to the influence of long steaming (heating). The longer the level of sterilization (heating), the greater the amount of heat received. According to Black's principle, the greater the amount of heat received, will be directly proportional to the large temperature change. The higher the temperature, the more microbes will die, thus minimizing the failure of pure culture. Sterilization levels 2 and 1 are less than optimal sterilization due to the short sterilization time so that there are still microbes contained in the PDA. The presence of microbes in PDA supports microbial growth during inoculation, making it less effective for producing pure cultures.

Table 2 Isolation Success Data (Tissue Culture) for Pure Culture (F0)

Repetition of-	Sterilization level		
	Level 1	Level 2	Level 3
1	✓	✓	✓
2	✓	✓	✓
3	✓	✓	✓
4	-	-	✓
5	-	-	-

Description:
✓ : isolation successful
- : isolation contaminated

After isolation, the tube containing mycelium is stored in a container at an optimal temperature for mycelium growth, around room temperature (25-29 °C) [11]. The higher the container's temperature, the more heat it distributes to the tubes containing mycelium. If the temperature used exceeds the room temperature range, the mycelium will be damaged; if it is below the room temperature range, the mycelium will not grow properly.

3.3. Spread Culture (F1)

The mycelium that has grown in the tube is cultured again into its second medium, namely sorghum media with a mixture of dextrose and sawdust. The results of this culture are called the seedlings (F1). Mycelium in one test tube can produce seedlings for 3 three bottles of spread seedlings. F1 is declared successful if, after about 3 weeks or 4 weeks, the bottle has been fully covered with fine threads or white mycelium. In this study, three repetitions were carried out for each level of sterilization with a total of nine repetitions, and all were successful as shown in Table 3. This is indicated

by the absence of contaminated mycelium. The success of all spread seedlings is due to the length of sterilization and the steaming temperature of the media (sorghum) when sterilisation is performed at the optimum temperature, namely 102^{o}C. In addition, it is also supported by the growth medium, namely sorghum. Sorghum, as a growth medium, provides the nutritional content needed for mycelium growth. As with F0, F1's success is also influenced by temperature. The temperature for mycelium growth is the optimum range of 25-29 °C, and the storage area is sterile and clean.

Table 3. Seedling Success Rate (F1)

Sterilization level	Repetition		
	1	**2**	**3**
1	✓	✓	✓
2	✓	✓	✓
3	✓	✓	✓

Description:
✓ : isolation successful
- : isolation contaminated

3.4. Planting Seed (F2)

After the mycelium in F1 has grown well, it is cultured into the planting seed medium (F2). The F2 media is a mixture of bran, sawdust, and water. One bottle of F1 can produce 15 bottles of F2 seeds. This F2 media will be used for cultivating white oyster mushrooms. The success rate of F2 is indicated by the growth of fine white threads for about 3 to 4 weeks. In this study, not all o2 progeny were successful, as shown in Table 4. However, the success rate is quite high. This is because the temperature in the F2 storage area is optimal for its growth (25-29 °C), and the planting seed media also resembles the cultivation media.

Table 4 Success Rate of Planting Seedlings (F2)

Sterilization level	Repetition	Result
1	1	✓
	2	✓
	3	✓
	4	✓
	5	✓
	6	✓
	7	✓
	8	✓
	9	✓
	10	✓
	11	✓
	12	✓
	13	-
	14	-
2	1	✓
	2	✓
	3	-
	4	-
	5	-
	6	-
	7	-

Description:
✓ : isolation successful
- : isolation contaminated

3.5. Mycelium Characterization using FTIR Method

If infrared radiation is applied to an organic compound sample, several frequencies can be absorbed by the compound. The number of frequencies that pass through the compound is measured as transmittance [12]. The magnitude of the transmittance intensity (%T) of the infrared spectrum absorption band at each wave number is equivalent to the number of functional groups in a sample tested by FTIR [13] When the transmittance reaches its maximum value, it does not indicate any vibration. Vibration occurs when a sample experiences maximum absorbance. Maximum absorbance indicates a large number of rays absorbed so that many molecules interact with each other and cause vibrations between molecules [14]. When the mycelium of each seed experiences maximum absorbance, stretching vibrations of C-O, C-N, C=O, C-H, and O-H are detected.

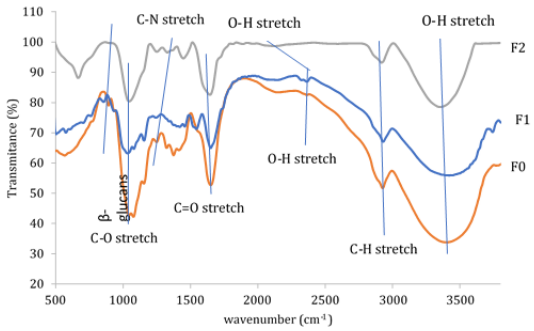

Figure 1. FTIR Characterization Results of F0, F1, and F2.

Based on the FTIR characterization results shown in Figure 1, the absorption bands formed from the mycelium of each seedling depict the same dominant absorption band pattern, only differing in the absorbance value. This shows that each seedling mycelium contains the same functional group. The difference lies in the β-glucan bond and the wave number value. The 1,3-β-D-glucan bond appears in pure culture, while in the scattered seeds and planted seeds the absorption band that appears is the 1,4-β-D-glucan bond.

The differences in β-glucan bonds among seedlings are thought to result from changes in the structure of functional groups from pure culture to scattered seeds, or from scattered to planted seeds. This is because the seeds are no longer purely from mushrooms, but have been mixed with sorghum and sawdust, so that in the scattered seeds and planted seeds, the 1,3-β-D-glucan peak does not appear, but what appears is the 1,4-β-D-glucan peak. The 1,3-β-D-glucan bond in F0 mycelium for each sterilization level is indicated by the presence of absorption bands at wave numbers 856 cm^{-1}, 856 cm^{-1}, 895 cm^{-1}, 894 cm^{-1}, and 894 cm^{-1}. According to the literature, the presence of a 1,3-β-D-glucan bond is indicated by the absorption band at 895 cm^{-1} [15].

The type of beta glucan from the mycelium of the spread and planted seedlings is indicated by the appearance of an absorption band of the 1,4-β-D-glucan bond at wave numbers 933 cm^{-1} and 1034 cm^{-1} for the spread seedlings, and 925 cm^{-1} for the planted seedlings. According to the literature, the presence of a 1,4-β-D-glucan bond is indicated by the absorption band at 930-1025 cm^{-1} [16].

Based on the above data, pure-culture mycelium, seedlings, and planted seedlings showed the presence of beta-glucan. Beta-glucan is the main component of polysaccharides found in the cell walls of white oyster mushrooms, which contain substances that can stimulate the immune system and are anti-cytotoxic, anti-mutagenic, and anti-tumorogenic compounds [17]. Infrared energy cannot excite electrons but can cause molecules to vibrate at specific vibrational levels.

This vibration phenomenon is used to detect functional groups (stretching vibrations) and to identify compounds and analyze mixtures (bending vibrations). In diatomic molecules, there is only one type of vibration: stretching. However, if a molecule contains many atoms, there will be many bonds, which means many types of vibrations.

The mycelium characterized by FTIR is modeled as a diatomic molecule, so that only stretching vibrations are analyzed in this study. The results of FTIR characterization provide information that mycelium contains C-O, C-N, C=O, C-H, and O-H functional groups. Mycelium contains protein indicated by the appearance of aromatic amine functional groups, namely C-N bond functional groups. Mycelium still contains a fairly high-water content, as indicated by the presence of O-H functional groups. Mycelium contains carbohydrates, indicated by the appearance of C-O, C=O, and C-H functional groups.

3.6. Optical Properties of Mycelium

The optical properties of the sample can be studied from the characterization results using FTIR by utilizing the Kramer-Kronig relation method. Based on the transmittance value and wavenumber, the refractive index value can be derived, namely the real refractive index (n) and the imaginary refractive index (k). The intersection graph of the n and k values at shorter wavelengths is called the Transversal Optics (TO). The intersection of the n and k values at longer

wavelengths is called the Longitudinal Optics (LO). Figures 2 and 3 show the LO and TO values for mycelium in the spread seedling media culture (F1) and planting seedling media (F2). The differences in media culture indicate that the LO and TO values in the shift from pre-adapted seedling culture to transplanting seedling culture shift towards a larger wavenumber. The LO value is observed at wavenumbers 590 and 905 cm^{-1} for both samples F0 and F1. While the TO value is at wave numbers 577 and 850 cm^{-1} for each sample F0 and F1. These results indicate that the energy required for mycelium growth in the spread seedling culture media is lower than in the planting seedling culture media. This is in accordance with the relationship between photon energy, which is directly proportional to the wave number.

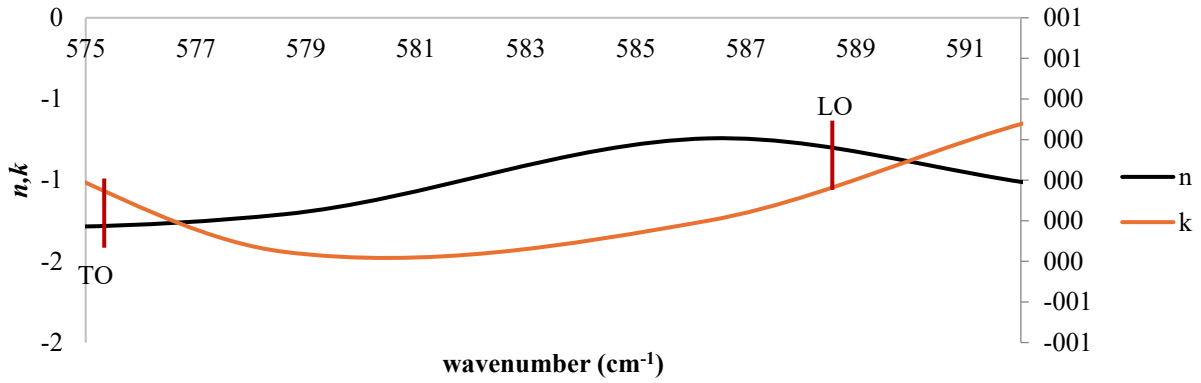

Figure 2. Results of LO and TO values of sample F1.

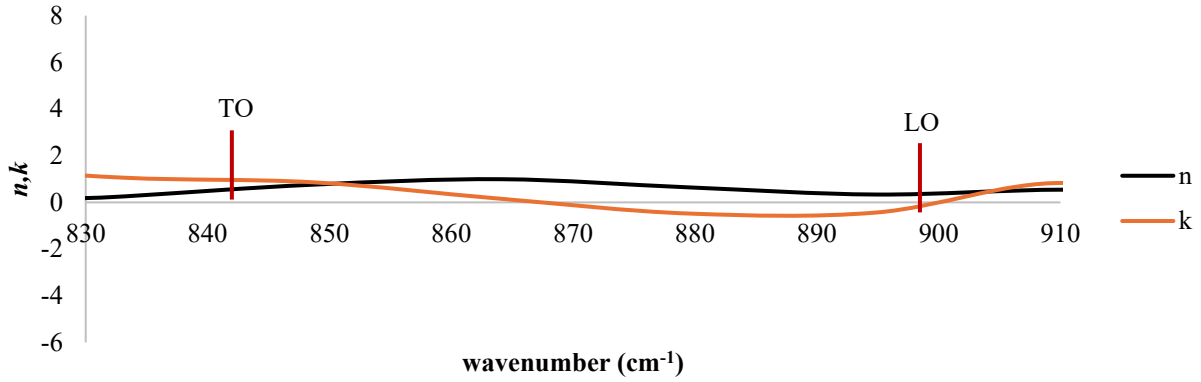

Figure 3. Results of LO and TO values of sample F2.

4. CONCLUSION

Based on the results of this study, it can be concluded that the third sterilization level had the most significant effect in the preparation of Potato Dextrose Agar (PDA) media, as all the produced spawn (F1) showed good growth, and no contamination was observed.

In addition, the LO and TO values of each sample were analyzed, showing that for both F1 (spread seeds) and F2 (planted seeds), the values shifted toward higher wavenumbers. This indicates that the photon energy required for the growth of F2 samples is greater than that for F1.

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