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Techniques for Preparing Spores and Hyphae of *Schizophyllum commune* for Morphological Observation

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ABSTRACT

At present, light microscopy (LM) and electron microscopy (EM) has been an indispensable tool to further our understanding of cellular and subcellular processes of microbiology. The aim of this study to have been used microscopy techniques to study the detail of investigating morphological characteristics of spores and hyphae of mushrooms such as the structure of the wall pattern, shape, and size of spores and hyphae of mushrooms. Observation morphological of *Schizophyllum commune* with a light microscope and transmission electron microscope. The experiment showed that the thickness of *Schizophyllum commune* for the cell walls, spores have an average of 258.83± 29.685 nm. Range 186.00-347.00 nm. and hyphae have an average of 228.25 ± 45.589 nm. Range 144.00-396.00 nm. Observation morphological of *Schizophyllum commune* with a scanning electron microscope showed that the hyphae and connected by hypha-clam connection, basidiospores are spores that are produced in a basidium cell division mitosis phase. However, the technique necessary used in combination with TEM with SEM to yield much better accuracy. As a result, the morphological characteristics of *Schizophyllum commune* can be used for species identification of this genus.Which knowledge on the morphological study of mushroom taxonomy and other applications mushroom tissues. In addition to the knowledge from this research, it can be applied for other scientific information such as agriculture, botany, and environmental science and the agro-food and pharmaceutical industry.

INTRODUCTION

Transmission electron microscopy (TEM) is a powerful tool for imaging the morphological characteristics of small structures such as spores and hyphae in *Schizophyllum commune*. Transmission electron microscopy (TEM) is a powerful tool for imaging the ultrastructure of microorganisms. morphological characteristics of small structures such as spores and hyphae in *Schizophyllum commune*. For microscope technique prepared for light and transmission electron microscopy can be divided into eight major steps: primary fixation, washing, secondary fixation, dehydration, infiltration with transitional solvents, infiltration with transitional resin, embedding, and cutting [1,3,4].

Scanning electron microscopy (SEM) has been used to study detail of microorganisms morphology such as the structure of the wall pattern, shape, and size of microorganisms. small structures such as spores and hyphae in *Schizophyllum commune.* The technique prepared for scanning electron microscopy can be divided into five major steps: primary fixation, washing, secondary fixation, dehydration, and drying in a critical point dryer. Technique prepared for scanning electron microscopy modified according to the method of B. L. Gabriel., 1982 [2]

Schizophyllum commune is a species of fungus in the genus Schizophyllum. it is an edible mushroom with high nutritional value. Moreover, mushrooms contain bioactive substances. There are a lot of anticancer and immunological activities, and in Korea, mushrooms are used to treat and prevent blood clots [7]. As shown in many previous studies, which is a typical local mushroom also comprises high nutritional and high bioactive compounds such as β-glucan. Schizophyllan, a β-glucan, was exhibited as a medicinal compound for anti-tumor, anti-cancer, anti-microbial, anti-viral, and anti-oxidant properties [6]. The aims, In addition to the knowledge from this research, it can be applied for other scientific information such as agriculture, botany, and environmental science and the agro-food and pharmaceutical industry.

METHODOLOGY

The mushroom grows on one side as a fan-shaped like 1-3 cm wide and 1-4 cm long, top white, dull, with fine hair of the same color. Bottom brown or reddish-brown looks like a fin, but narrow, slim, separated into lobes. Radius from the base of the fruiting body to the edge when dry, the edges will curl down. With a ripped mark in a long way, almost reaching the base of the flower somewhere making it look like a hook-and-drop hook spore shaped white oval, 3-4 x 1-1.5 µm, smooth surface [9,10] (Figure 1).

Figure 1. *Schizophyllum commune*.

Figure 2. *Schizophyllum commune* on PDA.

Ultrastructure under light microscopy

The mycelium on a petri dish incubated in an inverted position for 30 days at 25 C. were cut into small pieces (1x 2 mm) and then were fixed in primary fixative containing 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.2 for 12 hours at 4 ˚C. After that, the specimens were washed 3 times (10 min per time) in the same buffer. After washing, the specimens were post-fixed in 2 % osmium tetroxide in distilled water for 2 hours and then washed with distilled water 3 times (10 min per time). The specimens were dehydrated in a graded series of acetone 20 - 100 % (10 min per series) modified according to the method [4,8]. The specimens were then embedded in Spurr's low viscosity epoxy resin. After polymerization at 70˚C for 8 hours using a formulation of Spurr's resin (1969) [1] , the specimens were cut into pieces 1 µm thick using an ultramicrotome EM UC7 (Leica; Austria) with a glass knife, and mounted onto a glass slide. The specimens were stained in 1% Toluidine blue in borax at 85˚C for 1 minute and stained in 1% Basic Fuchsin in distilled water at room temperature for 2 minutes, then dried at 85˚C for 20 minutes, and closed with a cover slide [8]. Thirty areas per specimen were examined under light microscope (Carl Zeiss; Axiostar Plus), which was equipped with a photographic camera under normal bright-field imaging (Figure 6).

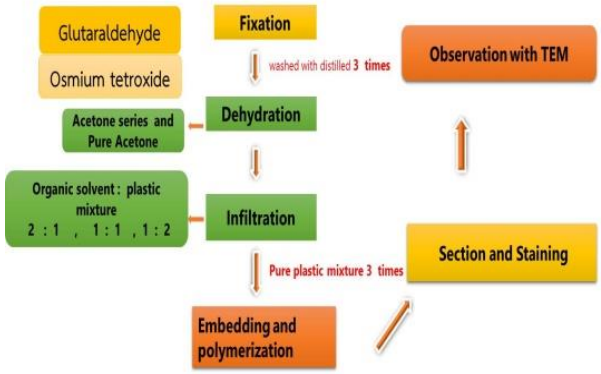

Figure 3. Preparation techniques for TEM.

Ultrastructure under Transmission Electron Microscopy

For transmission electron microscopy, the sample preparation was the same as that of light microscopy. Nevertheless, Ultrathin section (60 nm) specimens were prepared using a Leica ultramicrotome EM UC7 (Leica; Austria), with a diamond knife, and the specimens were mounted on copper grids. The specimens were stained with 5% aqueous uranyl acetate for 15 minutes and Reynold's lead citrate for 15 minutes [3] (Figure 3,5) and examined under an HT7700 (Hitachi; Japan) with a high voltage of electron at 80 keV.

Ultrastructure under Scanning Electron microscopy

The mycelium on a petri dish incubated in an inverted position for 30 days at 25 C. were cut into small pieces (5x 5 mm) and then were fixed in primary fixative containing 2.5 % glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.2 for 12 hours at 4 ˚C. After that, the specimens were washed 3 times (10 min per time) in the same buffer. After washing, the specimens were post-fixed in 1 % osmium tetroxide

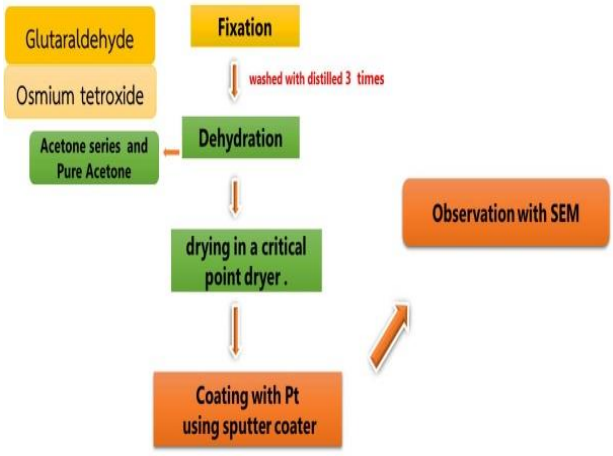

Figure 4. Preparation techniques for SEM.

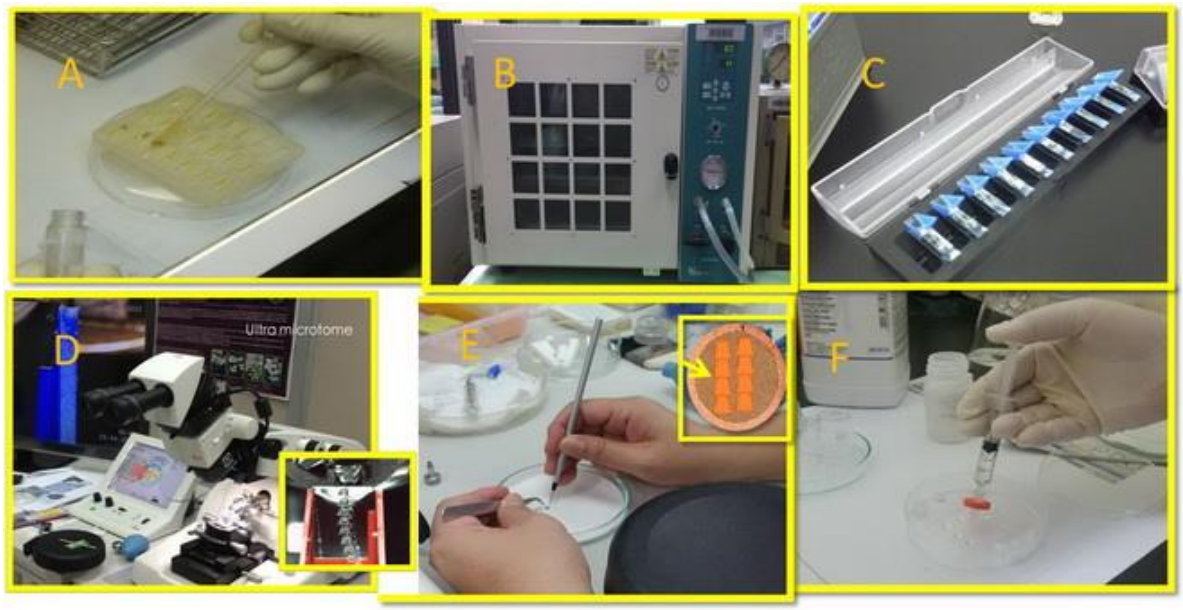

Figure 5. Sample Preparation for Transmission Electron Microscope. A-B. Infiltrating Spurr's resin into specimen and polymerization in vacuum oven pump. C. Glass Knife. D-F. Preparation of ultrathin section with ultramicrotome and collection of thin section on grid (arrow) and double staining with uranyl acetate and lead citrate.

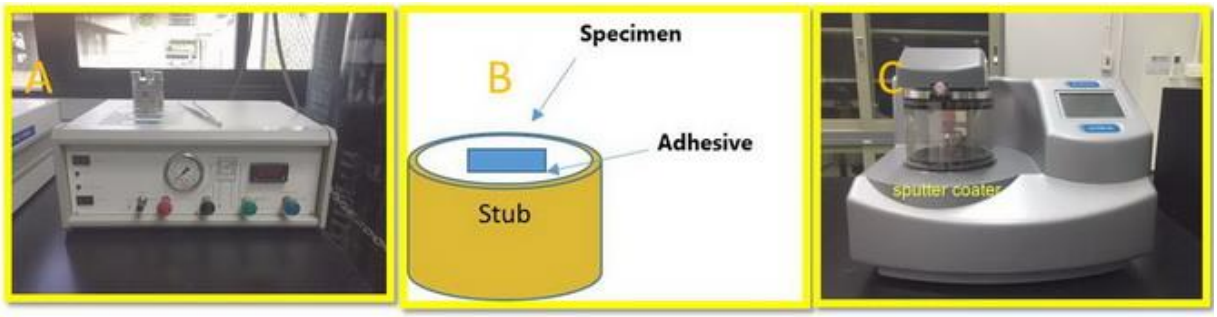

Figure 6. Sample Preparation for Scanning Electron Microscope. A. Drying the sample at the critical point drying by importing the critical point dryer B. Preparation samples on stub were coat with platinum in Sputter Coater. C. Samples were coated with platinum in sputter coater (Quorum; Q150RES).

in distilled water for 1 hours and then washed with distilled water 3 times (10 min per time). The specimens were dehydrated in a graded series of acetone 20 - 100 % (10 min per series) modified according to the method and dried in a critical point dryer (Quorum; K850) [3] (Figure 4,6). Samples were coated with platinum in sputter coater (Quorum; Q150RES) and examined in Field Emission Scanning Electron Microscope analysis (Hitachi; SU 8020) (Figure 4).

RESULTS AND DISCUSSION

The morphology of colonies in the study of the development of spore mushroom, *Schizophyllum commune* has created a circular shape with elevation like an umbonate, with a small convex button in a circle. In the center of the colony. The colony has two curled margins. The inner circle is wider than the outside, narrower than the white colony. The inner circle is in the center of the colonies with longer fibers. The outer surface of the substrate has less fiber. The fibers have a long, fine detail. The inner circle is denser than the outer circle [10,11] (Figure 2).

The experiment showed that results from the measurement thickness of cell wall spore, hyphae of Schizophyllum commune have an average of 258.83± 29.685 nm. Range 186.00-347.00 nm. and hyphae have an average of 228.25 ± 45.589 nm. Range 144.00-396.00 nm. in Table 1. Morphological observation of *Schizophyllum commune* with an SEM showed that the hyphae and connected by hypha-clam connection, basidiospores are spores that are produced in a basidium cell division mitosis phase. However, the technique necessary used in combination with TEM with SEM to yield much better accuracy. As a result, the morphological characteristics of *Schizophyllum commune* can be used for species identification of this genus. which knowledge on the morphological study of mushroom taxonomy and other applications mushroom tissues(Figure 7B-7D).

The protocol for sectioning of spores and hyphae of mushrooms using resin embedding. This protocol provides a reliable platform to

Table 1. Showing the thickness of cell wall spore, hyphae of *Schizophyllum commune.*

thickness of cell wall spore, hyphae (nm)			
spore		**hyphae**	
Range	Average	Range	Average
186.00-347.00	258.83±29.685	144.00-396.00	228.25±45.589

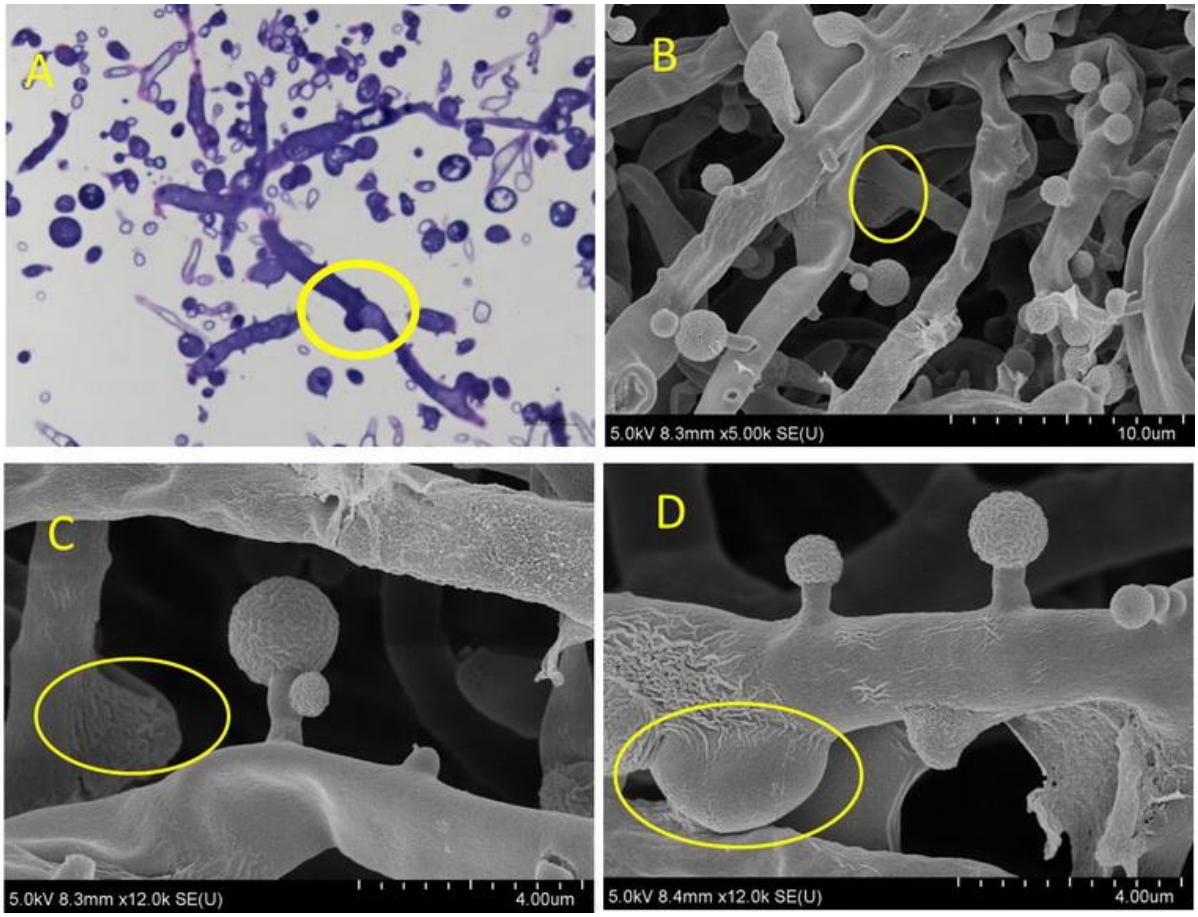

Figure 7. Photomicrographs and micrographs of *Schizophyllum commune* morphology structure. A. Light micrograph of Schizophyllum Commune morphology structure Showing characteristics hypha-clam connection (circle) (100x) Bar 10 µm. B. Scanning Electron microscopy micrograph of morphology showing structure characteristics hypha-clam connection (circle) (magnification 5,000 X) Bar 10 µm. C. Scanning Electron microscopy micrograph of morphology showing structure characteristics hypha-clam connection (circle) (magnification 12,000 X) Bar 4 µm. D. Scanning Electron microscopy micrograph of morphology showing structure characteristics hypha-clam connection (circle) (magnification 12,000 X) Bar 4 µm.

yield high-quality images of cross-sections allowing the study of the development of various tissue layers across the transversal axis and long of spores and hyphae of mushrooms. As this method is an adaptation of the protocol developed for spores and hyphae of mushrooms [1,5,7], it can easily be modified to accommodate other organs and simple. Spurr's Low Viscosity embedding mixture is recommended because of its excellent penetration qualities, which provide good and rapid infiltration of tissues [1,5].

CONCLUSION

In conclusion, the application of microscopic and electron microscopy techniques to study morphology in the study of the size, shape, and structure of microbes. The results of this study have shown the combination of microscopic and electron microscopy techniques can study the microstructure of both external and internal cells. *Schizophyllum commune* very well. (Figure 7,8 and 9)

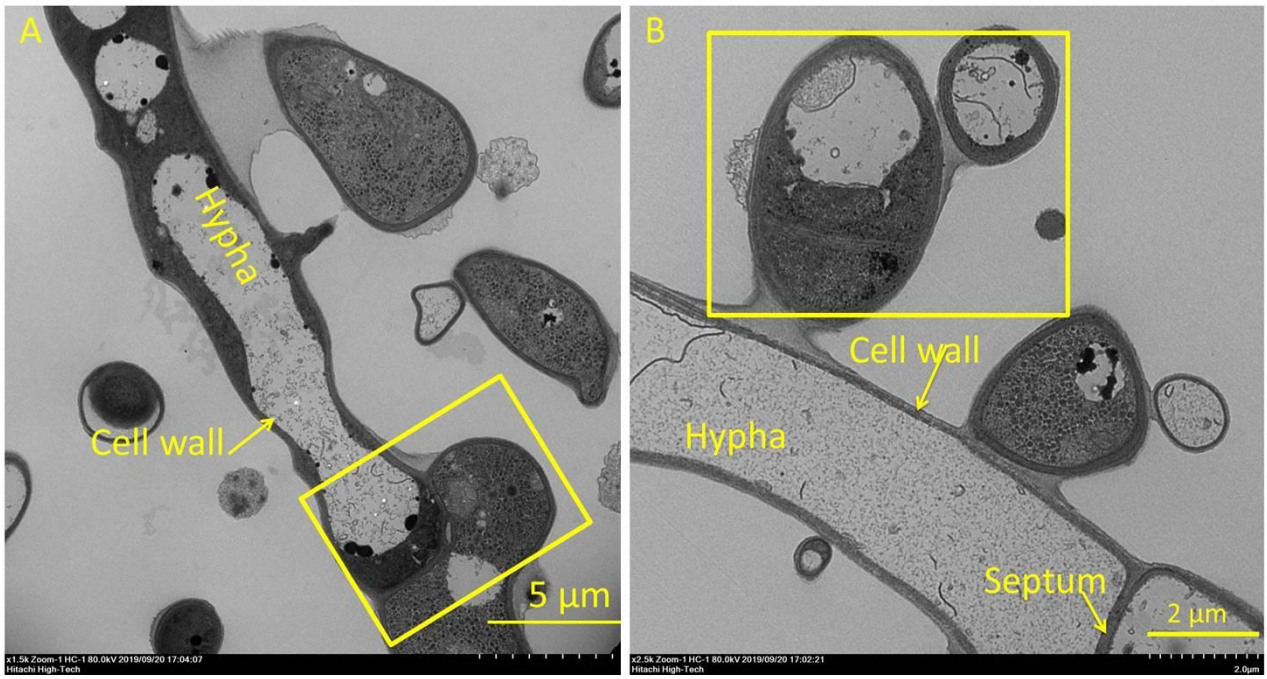

Figure 8. Photomicrographs and micrographs of *Schizophyllum commune* morphology structure. A. Transmission Electron microscopy micrograph of *Schizophyllum commune* morphology structure Showing characteristics hypha-clam connection (square) hypha, cell all (magnification 15,000 X) Bar 5 µm. B. Transmission Electron microscopy micrograph of *Schizophyllum commune* morphology structure Showing characteristics hypha,septum and basidiospores are spores that are produced in a basidium cell division mitosis phase (square) hypha, cell all (magnification 25,000 X) Bar 2 µm.

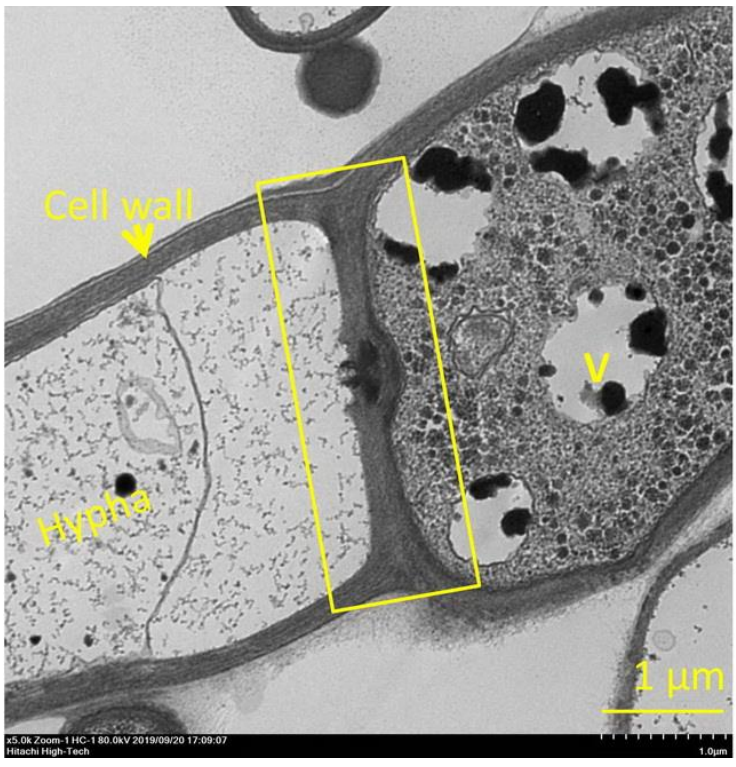

Figure 9. Photomicrographs and micrographs of *Schizophyllum commune* morphology structure. A. Transmission Electron microscopy micrograph of *Schizophyllum commune* morphology structure Showing characteristics septum (square) hypha, cell wall (arrow) and V,vacuole. (magnification 5,000 X) Bar 1 µm.

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