Microsc. Microanal. Res. 2021 34(1) 13-18

Microscopy and Microanalysis Research
- The Journal of The Microscopy Society of Thailand

Journal Home Page: https://www.tci-thaijo.org/index.php/mmres/

Microsc. Microanal. Res.

Ultrastructural Studies of *Ganoderma cf. Lucidum* Cell by Microscopic Technique.

Patcharee Umroong[1]*, Yupadee Paopun[1] and Yaovapa Aramsirirujiwet[2]

[1] *Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, Kasetsart University, Bangkok, 10900, Thailand.*
[2] *Department of Microbiology, Faculty of Science, Kasetsart university, Bangkok, Thailand.*
*Corresponding author's e-mail address: : rdipru@ku.ac.th

ARTICLE INFO

Article history
Submitted: 12 April 2021
Revised: 17 June 2021
Accepted: 23 June 2021
Available online: 29 June 2021

Keywords:
Ultrastructure; Microscopy; Transmission Election Microscopy; Scanning Electron Microscopy; *Ganoderma* cf. *lucidum*

ABSTRACT

Microscopic techniques have been applied to study the details of hyphae, spores of mushroom morphology for mycelia growth cultured in potato dextrose agar (PDA) such as the structures of the cell wall pattern, shape, and size of spores and hyphae of mushroom. The examination with transmission electron microscopy, showed that the cell wall thickness of *Ganoderma* cf. *lucidum*, spores has an average of 198.5± 105.7 nm. Range 82.9-438.0 nm. and hyphae have an average of 211.3 ± 122.6 nm. Range 59.90-508.00 nm. Morphological studies of *Ganoderma* cf. *lucidum* mushroom with scanning electron microscopy and optical microscopy showed the characteristics of the hyphae and hypha-clam connection of cultured cells. Ultrastructural studies using microscopic techniques are suitable to study the morphology of *Ganoderma* cf. *lucidum* as the basics for further research. In addition to knowing information from this research, it can be applied to other research such as biodiversity, botany as well as information on the food and pharmaceutical industries at present.

INTRODUCTION

The techniques involved microscopy and electron microscope, were used to study the details of microbial morphology such as structural features of cell wall patterns. This article discusses applications for the study of spore and fibrous morphology in *Ganoderma* cf. *lucidum* after cultured in potato dextrose agar (PDA), aged 31 days, incubated at 25 ° C by Asst. Prof. Dr. Yaovapa Aramsirirujiwet from the laboratory of Department of Microbiology, Faculty of Science Kasetsart University. Additionally, this article discusses optimal sample preparation for respecting both the structure and preservation, and preparation techniques for high-resolution study

A Compound Microscope is a type of microscope that commonly uses visible light and a system of lenses to generate magnified images of small and or thin objects. A compound microscope is equipped with a photographic camera under normal bright-field imaging camera and is typically used to capture images in two dimensional micrographs.

Transmission Electron Microscope (TEM) is a high-resolution camera and can distinguish it at a nanometer level. This is used for study of morphological microstructures, both externally and internally. The sample must be thin enough for the electron beam to pass through, because the image obtained by the refraction or deflection of the electron beam passing through the sample at different angles causes the difference in the intensity of the electrons present on the relay unit. The resulting image is two-dimensional.

Scanning Electron Microscope (SEM) is an advanced scientific instrument used to study the morphological microstructure on the surfaces of a sample. The essence of the sample must be free from moisture. The resulting image is three-dimensional.

Therefore, appropriate sample preparation methods take into account the structure and sampling for microscopic studies and electron microscopy. Additionally, appropriate sample preparation techniques to be able to fully study the morphological microstructure of the sample and the internal cell structure are perfect.

MATERIALS AND METHODS

Morphology

Ganoderma cf. *lucidum* after cultured in potato dextrose agar (PDA), aged 31 days, incubated at 25 ° C from the laboratory of Department of Microbiology, Faculty of Science Kasetsart University. (Figure 9A). The growth of *Ganoderma* cf. *lucidum* produces circular shape, umbonate elevation, with small circular bumps. In the center of the colonies, the colonies have 3 curled margins. The width of the circles is similar in size. All 3 white colony colonies. The inner band is in the middle of the colony with shorter mycelia. The surface is quite rough, not smooth. The middle and periphery of the surface are slightly very fluffy, but not very fluffy, fine mycelia. The mycelia have uniform density. This study, Microscope and Electron Microscope technique are applied for study of the morphology of *Ganoderma* cf. *lucidum.*

Compound Microscope

Morphology of *Ganoderma* cf. *lucidum* under a compound microscope

Preparation of *Ganoderma cf. lucidum*

In this study, a needle was used to scoop a sample the hyphae of mushroom on the cultured in potato dextrose agar (PDA) by a medium, placed on a glass slide with a drop of distilled water, covered with a glass lid, and then examined with compound microscope (Carl Zeiss; Axiostar Plus) mounted on the camera under normal brightness shooting modified from [5] (Figure 1)

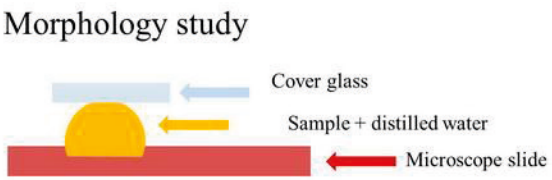


Figure 1. Shows the procedure for preparing samples for the compound microscope.

Preparation of *Ganoderma* cf. *lucidum* to study ultrastructures. Fixation and Dehydration

In this study, we described the double fixation technique. The mycelium groups growing on PDA were cut into two groups: the first group was cut into small pieces (1×2 mm), the second group was cut into small pieces (3×3 mm) from the petri dish. They 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. Primary fixation: Glutaraldehyde is the most effective substance in maintaining the fine structure of cells. The first group was for microscope and transmission electron microscopy studies and the second group was for scanning electron microscopy studies. The specimens were washed 3 times (10 min per time) in the same buffer. After washing, the specimens were placed in a secondary fixative in 2 % osmium tetroxide in distilled water for 2 hours and then washed with distilled water 3 times (10 min per time). Secondary fixation: Osmium tetroxide is a substance that reacts to many cellular elements, cross-link lipid with double bond showing in (Figure 9B). The specimens were dehydrated in a graded series of acetone 20 - 100 % (10 min per series). Follow the method of Patcharee et al. 2020. [6] (Figure 2).

Infiltration

After the dehydration procedure, the first cell group the sample must have a replacement in the cell by infiltration. The composition inside the cell with organic solvent: plastic mixture. Here we use acetone to extract water from the cell because it is soluble and compatible with plastic. Thus, we can do the infiltration step by reducing the amount of acetone proportionally, while gradually increasing the amount of plastic mixed by using Acetone: Spurr's Resin (2 : 1, 1 : 1, 1 : 2) and Pure Spurr's Resin 3 times. The first cell group is replaced with a low viscosity epoxy resin when completely replaced (Figure 2).

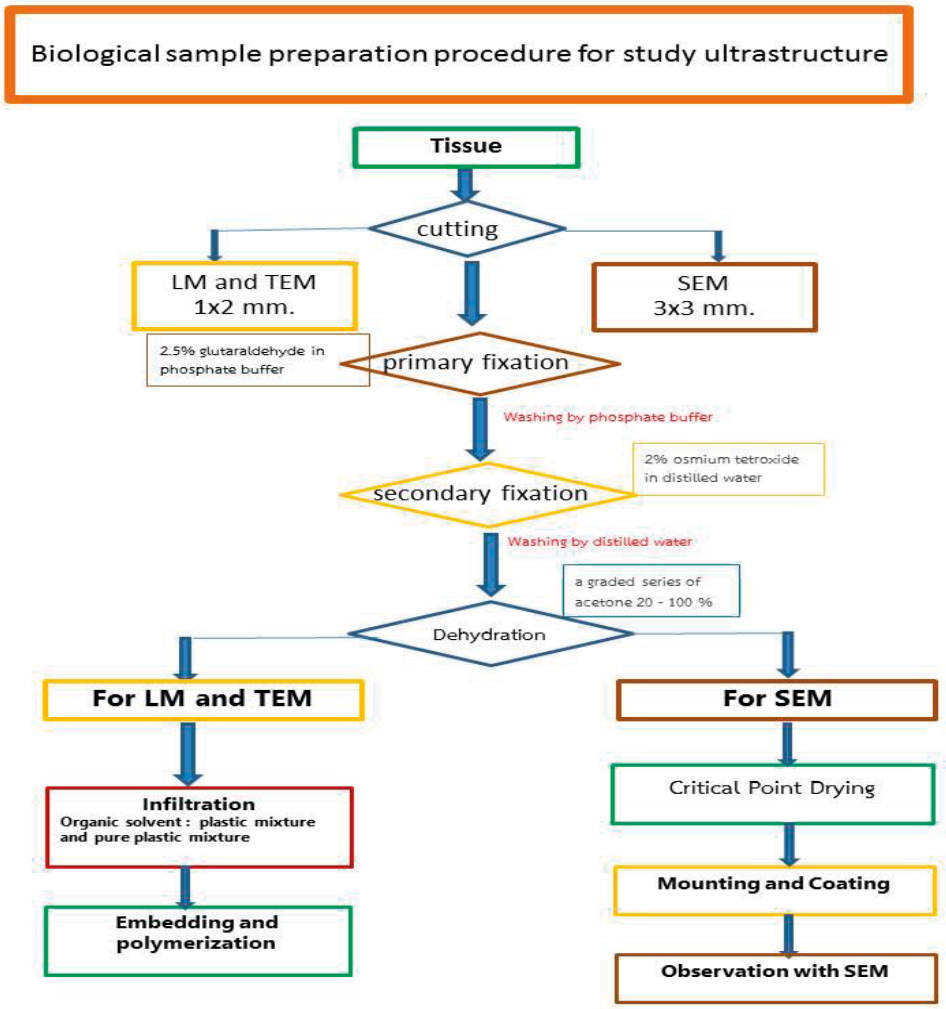


Figure 2. Showing biological sample preparation procedure for study ultrastructure.

Embedding and polymerization

The samples were put into flat embedding molds and then put in low viscosity epoxy resin. The samples were then polymerized at 70 ˚C for 8 hr. in the vacuum oven pump using the recipe of Spurr's resin (1969) [1] (Figure 3).

Component	Formula(g)
Vinyl cyclohexane dioxide(ERL 4221)	10 g
Diglycidyl ether of Polypropyleneglycol (DER® 736)	8 g
Nonenyl succinic anhydride (NSA)	25 g
Dimethylaminoethanol (DMAE)	0.4 g

Figure 3. Showing preparation procedure recipes for Spurr's resin.

Sectioning and staining sample.

The semi - thin and Ultra-thin sample cutting at the nanometer scale requires an instrument called an ultra microtome. In this step, we divide it into two steps: first step specimens were cut into pieces 1 µm thick using an ultra microtome 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 and closed with a cover slide [4]. The specimen were examined under light microscope (Carl Zeiss; Axiostar Plus), which was equipped with a photographic camera under normal bright-field imaging (Figure 4, Figure 5and Figure 6)

Second step For transmission electron microscopy in this step specimens were cut into pieces Ultrathin section pieces (90 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 for cell wall thickness of spores and hyphae were measured. under an HT7700(Hitachi; Japan) with a high voltage of electron at 80 keV (Figure 4, Figure 5and Figure 6).

Morphology of *Ganoderma* cf. *lucidum* under scanning electron microscopy

Ultrastructure under Scanning Electron microscopy

For second group were scanning electron microscopy studies the sample preparation was the same as that of compound microscopy and

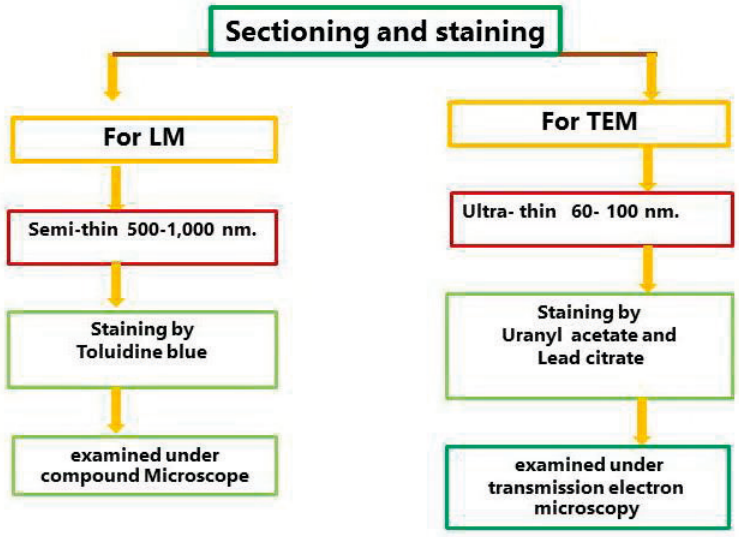
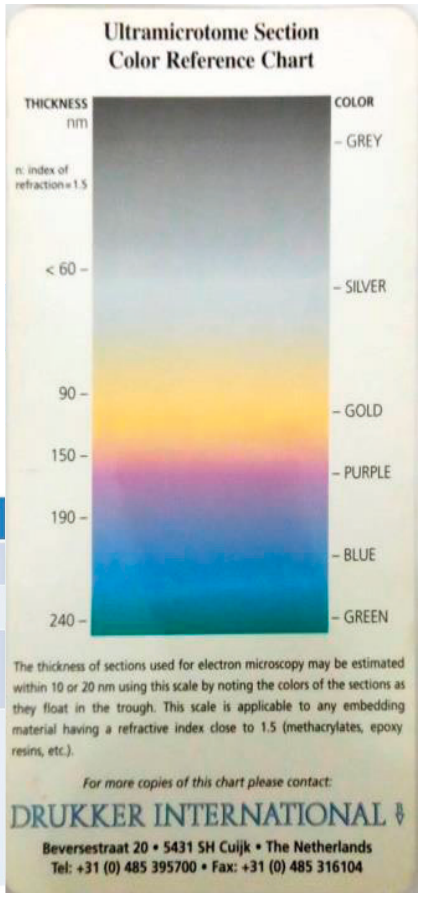


Figure 4. Showing sectioning and staining.

color	interactions
Uranyl acetate	Interactions with nucleic acids and proteins.
Lead citrate	Interactions with phospholipids and Glycogen.
Toluidine blue	Interactions with nucleic acids and proteins. Resulting in blue, the nucleus is dark blue.

Figure 5. Showing interactions of color.

Color	Thickness(nm)	Purpose
grey	<60	For high resolution
silver	60-90	Useful for most purposes
gold	90-150	Low magnification and autoradiography only
purple	150-190	Too thick
blue	190-240	
green	240-280	
yellow	280-320	



Ultra microtome Section Color Reference Chart

Figure 6. Showing ultra microtome section color reference chart.

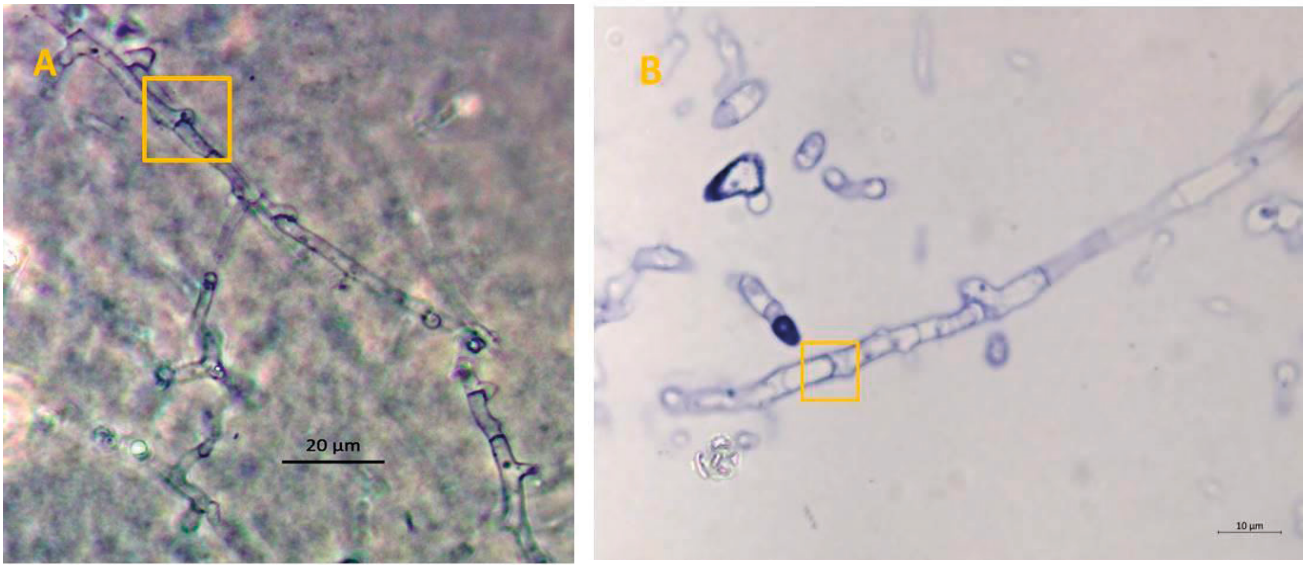


Figure 7. Photomicrographs of *Ganoderma* cf. *lucidum* morphology structure.
A: optical microscopy images of *Ganoderma* cf. *lucidum* showing characteristics of the hyphae and hypha-clam connection of cultured cells. (Squares) by wet mount techniques (magnification 40 X) Bar 20 µm.
B: optical microscopy images of *Ganoderma* cf. *lucidum* showing characteristics of the hyphae and septum of cultured cells. (Arrow) ultrastructures techniques thick 1 µm. (magnification 100 X) Bar 10 µm.

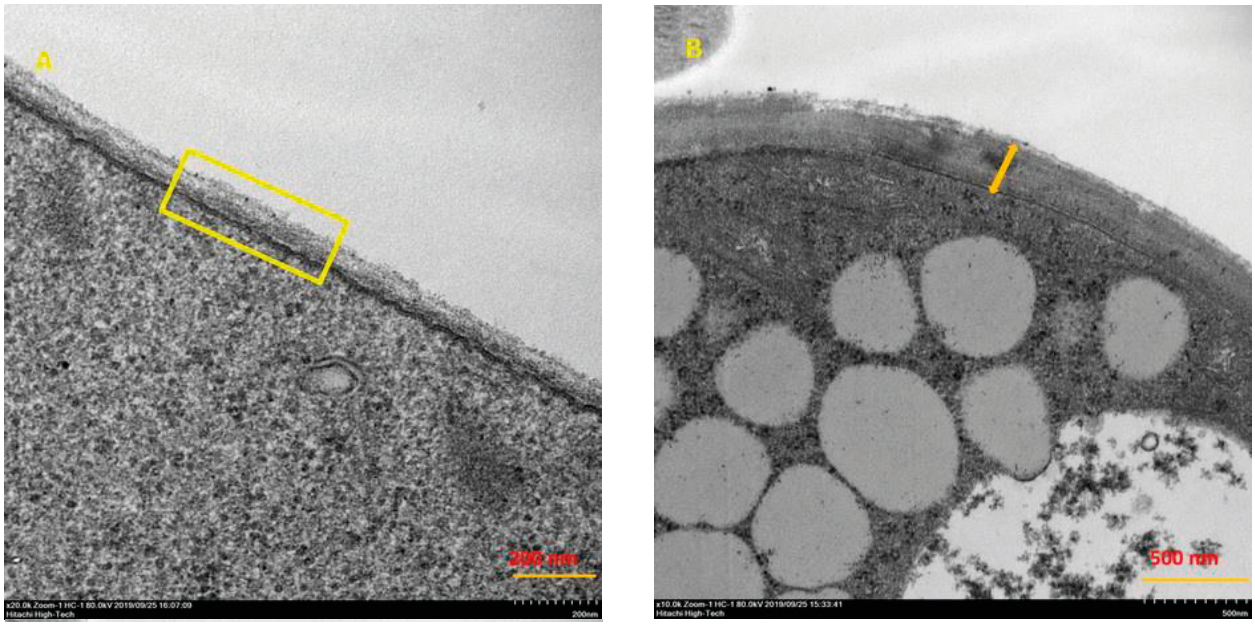


Figure 8. Photomicrographs of *Ganoderma* cf. *lucidum* morphology ultra structure.
A: Transmission electron microscopy images of *Ganoderma* cf. *lucidum* showing cell wall of hyphae (Squares) (magnification 20,000 X) Bar 200 nm.
B: Transmission electron microscopy images of *Ganoderma* cf. *lucidum* showing cell wall of spores (Squares) (magnification 10,000 X) Bar 500 nm.

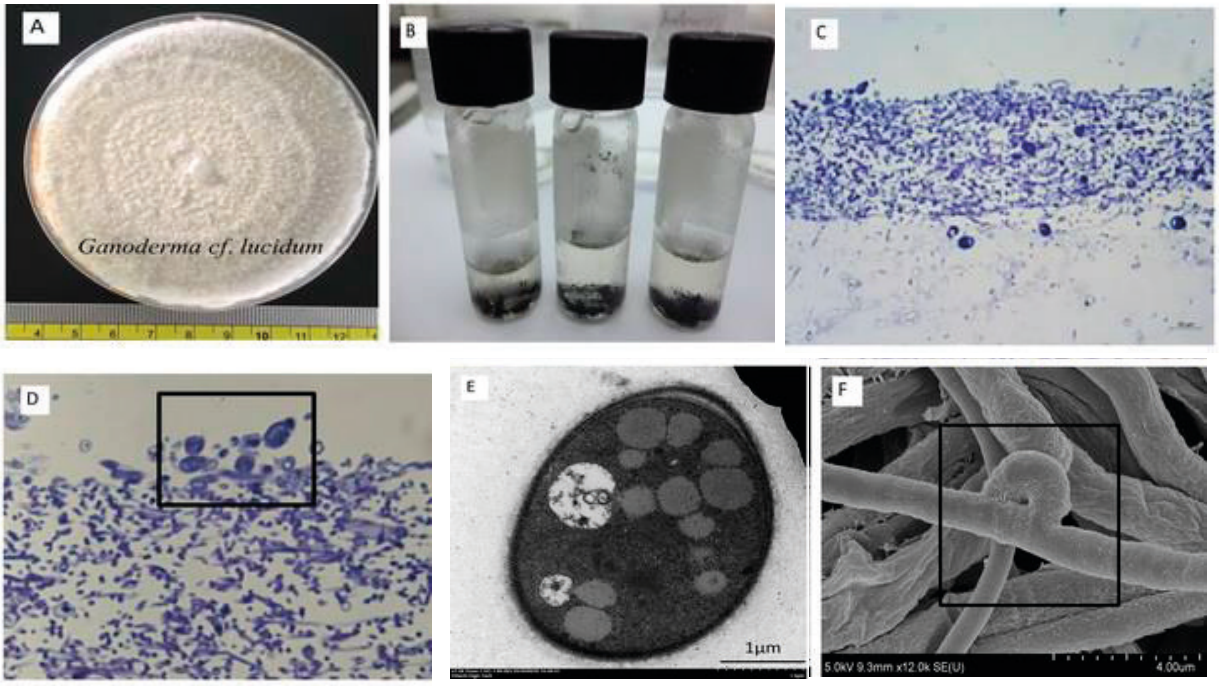


Figure 9. Photomicrographs and micrographs of *Ganoderma* cf. *lucidum* morphology structure.
A: Showing *Ganoderma* cf. *lucidum* grown on PDA.
B: Showing demonstration of fixatives on osmium tetroxide are stored in glass stoppered.
C: Light microscopy images of *Ganoderma* cf. *lucidum* l showing spores and hyphae (Squares) (magnification 40 X) Bar 20 µm.
D: Light microscopy images of *Ganoderma* cf. *lucidum* showing spores (Squares) (magnification 100 X) Bar 10 µm.
E: Transmission electron microscopy images of *Ganoderma* cf. *lucidum* showing spores (magnification 7,000 X) Bar 1µm.
F. Scanning electron microscopy micrograph of *Ganoderma* cf. *lucidum* morphology showing structure characteristics hypha- clam connection (Squares) (magnification 5,000 X) Bar 4 µm.

transmission electron microscopy. The specimens were dehydrated in a graded series of acetone 20 - 100 % (10 min per series) after that we modified it according to the method and dried it in a critical point dryer (Quorum; K850) [2]. Samples were coated with platinum in a sputter coater (Quorum; Q150RES) and examined in field emission scanning electron microscope analysis (Hitachi; SU 8020) (Figure 2). [2]. Samples were coated with platinum in a sputter coater (Quorum; Q150RES) and examined in field emission scanning electron microscope analysis (Hitachi; SU 8020) (Figure 2).

RESULTS AND DISCUSSION

When viewed by optical microscopy, they show on hyphae Figure 7 A and 7B is micrograph the characteristics of the hyphae and septum and hypha-clam connection of cultured cells while Figure 9C and 9D is morphology characteristics in mycelia growth cultured in potato dextrose agar (PDA). While the thickness of the cell wall spores and hyphae were examined with transmission electron microscopy shown in Figure 8A and 8B and image the resulting image is two-dimensional. Figure 9F is a scanning electron microscopy micrograph showing characteristics of the hyphae and hypha-clam connection of cultured cells and the image the resulting image is three-dimensional.

CONCLUSION

The experiment showed that results using Transmission electron microscopy the measurement thickness of cell wall spore, hyphae form of *Ganoderma* cf. *lucidum*, spores have an average of 198.46 ± 105.678 nm. Range 82.90-438.00 nm. and hyphae have an average of 211.26 ± 122.562 nm. Range 59.90-508.00 nm. Morphological observation of *Ganoderma* cf. *lucidum* with an SEM and LM 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 is necessary to be used in combination with microscopic techniques to yield much better accuracy. Ultrastructural studies using microscopic techniques are suitable to study the morphology of *Ganoderma* cf. *lucidum* as the basis for further research. In addition to knowing information from this research, it can be applied to other research such as biodiversity, botany, agriculture, and environmental science, as well as information on the food and pharmaceutical industries at present (Figure 9A-9F).

ACKNOWLEDGEMENT

The financial support provided by Kasetsart University Research and Development Institute, Bangkok, Thailand is gratefully acknowledged.

REFERENCES

[1] Spurr A.R., A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research*, **1969**, 26, 31-43.

[2] Gabriel B.L., *Biological Electron Microscopy*. Van Nostrand Reinhold Company Inc., New York. **1982**.

[3] Reynolds E.S. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology*. **1963**, 17, 208–212.

[4] Bozzola J., Electron Microscopy Principles and Techniques for Biologists, 1992.

[5] Umroong P. and Kotepong P., Ultrastructure studies of fungus *Colletotrichum gloeosporioides* cause of Anthracnose disease of mango by microscope technique. *Microsc. Microanal Res*. 2019. Vol.32 (2), 1-5.

[6] Umroong P, Paopun Y, Aramsirirujiwet Y , Sangsawang J, Techniques for Preparing Spores and Hyphae of *Schizophyllum communefor* Morphological Observation *Microsc. Microanal. Res*. 2020.Vol.33(1), 22-27

[7] Royal Institute. 2007. Musrooms in Thailand, *Royal Institute Edition 2nd edition*, Pimlak, Bangkok.