Isolation and Mycelium Growth of *Ganoderma lucidum* on *Manihot Esculent* Substrate with Mineral Supplement

Nguyen Thi Tuyet Nhung
University of Food Industry (HUFI), Ho Chi Minh City, Vietnam
Email: nhungntt@cntp.edu.vn

***Abstract*—Isolation to create a strong mycelium, young mycelium source is a very important factor in transplant of Ganoderma lucidum. Isolation to create Ganoderma lucidum mycelium in PDA (Potato Dextrose agar), isolation to create G.lucidum mycelium in brown rice, G.lucidum mecylium in grain with 5 (g) rice bran and $MgSO_4$ different ratios $CaCO_3$. Ganodrema lucidum using wheat bran supplemented with 4 grams rice bran and $MgSO_4$ at different concentrations significantly influenced the growth of Ganoderma lucidum mycelium on Manihot Esculenta Substrate.**

Index Terms*—isolation, mycelium growth, *Ganoderma lucidum

I. Introduction

Ganoderma lucidum is one of the most extensively studied mushrooms due to its edicinal properties [1], Ganoderma lucidum fruiting body is called 'Reishi' in Japanese and 'Lingzhi' in Chinese [2], [3]. Ganoderma lucidum revealed similar radial growth in both MMN and PDA media [4], Sawdust is the most preference main ingredient used in substrate mixtures for Ganoderma lucidum cultivation [5]. Ganoderma lucidum is a popular medicinal mushroom in Asia: China, Korea, Vietnam,... Isolate directly from the Ganoderma lucidum for the purpose of create young mycelium, healthy mycelium. The Ganoderma lucidum mushroom was cultured on the basis of Manihot esculenta with mineral supplementation with different levels from which the different mycelium growths of Ganoderma lucidum.

II. Matherial and Methods

A. Isolation of Ganoderma lucidum

PDA (Potato Dextrose agar) is conventional media used in mushroom culture contains

Potatoes 200 g, 20 g glucose, 20 g agar, 1 liter distilled water, KH_2PO_4 3 g, $MgSO_4$. $7H_2O$ 3g, vitamin B1 15 mg.

How to proceed: peel potatoes 200 g, washed, chopped, boiled within 20 - 30 minutes. Water filter for cooling, for 20 g of sugar, 20 g of agar. Heat to dissolve agar. Up to 1,000 ml of distilled water is distributed equally to the 18x150 mm test tubes, impervious cotton and test tube cover paper. Carefully pack the test tubes containing the PDA to autoclave at 121^0 C and pressurize at 1 atm for 30 minutes then let agar slope, let cool for 1 day then proceed to isolate.

Ganoderma lucidum is isolated to select the fungus with white edges about 0,5 cm. After selecting the fungus suitable for isolation, remove the stalk and mushroom Use alcohol 98^0 to clean the outer fungus, manipulate the cabinet under sterile conditions. Use a knife to cut a thin layer above and below the fungus to the source of infection of the sample, then cut the mushroom pieces into the test tube, the size of flesh mushroom about 0,25 cm^2. Then follow up the mycelium begins to grows.

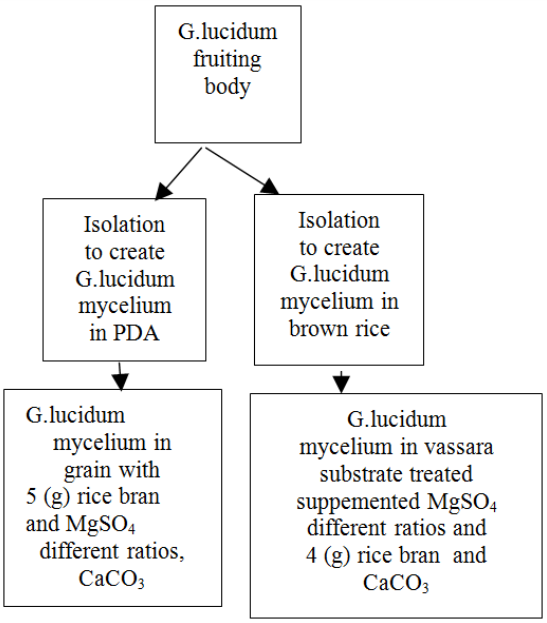

Figure 1. The step to creat G.lucidum mycelium

B. Isolation Created Ganoderma lucidum Mycelium on Brown Rice

Brown rice with a thick layer of bran including starch, fat, protein, fiber and vitamins such as B1, B2, B3, B6; acids such as paraaminobenzoic (PABA), pantothenic (vitamin B5), folic (vitamin M), phytic. The trace *elements such as calcium, selenium, glutathione (GSH),* iron, magnesium, potassium and sodium.

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How to proceed: 500 g of brown rice is soaked in water for 1,5 hours. Next to 1 liter of water to cook 100^0 C 45 minutes into rice, pour out the rice and bring to the sun 2h. Then, put the dried rice in a 100 ml glass bottle, wrap it and bring it to steam at 121^0C, 1atm, 30 minutes. Let cool for 1 day then proceed to isolate. G.lucidum with 0,5 cm white margin, mushroom stalk and 98 degrees of ethanol are used to clean the outer fungal ears. The isolation is performed under sterile conditions.

Use a scalpel to cut the mushroom size 0,25cm^2 into a glass bottle of brown rice.

Then track the stage grow Ganoderma lucidum mycelium and raise Ganoderma lucidum mycelium at temperature 22-30^0C.

C. *Effect of MgSO4 on Growth G.lucidum Mycelium with Supplementation of 5 (g) Rice Bran on Grain*

How to proceed: wash rice, remove loose seeds. Soak in 1% lime water soak for 8-12 hours, then rinse with clean water.

Put in the cooking pot until the grain of rice swells, then pick out to dry sun drying for 3-4 hours for dry rice

Combine the rice bran and MgSO4 in the ratio of survey:

R0 contents 100 g grain + 0 g $MgSO_4$ + 1g $CaCO_3$ + 5 g rice bran.

R1 contents 100 g grain + 0,1 g $MgSO_4$ + 1 g $CaCO_3$ + 5g rice bran.

R2 contents 100 g grain + 0,2 g $MgSO_4$ + 1g $CaCO_3$ + 5g rice bran.

R3 contents 100 g grain + 0,3 g $MgSO_4$ + 1 g$CaCO_3$ + 5g rice bran.

R4 contents 100 g grain + 0,4 g $MgSO_4$ + 1g $CaCO_3$ + 5g rice bran and put into glass bottles of 100 ml. Close the packaging and label.

Sterilize at 121^0C, 1atm, for 30 minutes for 1 day, transplant the fungus into and G.lucidum mycelium rearing at 30 - 32^0C. After 2 days of measurement G.lucidum mycelium.

D. *Research of the Effect of Mineral MgSO4 on the Mycelium Growth of Ganoderma lucidium on Manihot esculenta (cassava) Substracte*

Freshly shredded cassava, cut into 10 cm short sections, split into 4 equal parts dried, then soak $CaCO_3$ concentration 0,5% in 24 hours. After, wash with water and make dry. Manihot esculenta has been supplemented with 4 grams of rice bran and 1 gram $CaCO_3$ and minerals $MgSO_4$ according to the treatments.

A content of 20 grams cassava + 4 grams rice bran + 0 gram $MgSO_4$ + 1 gram $CaCO_3$.

B content of 20 grams cassava + 4 grams rice bran + 0,1 gram $MgSO_4$ + 1 gram $CaCO_3$.

C content of 20 grams cassava + 4 grams rice bran + 0,2 gram $MgSO_4$ + 1 gram $CaCO_3$.

D content of 20 grams cassava + 4 grams rice bran + 0,3 gram $MgSO_4$ + 1 gram $CaCO_3$.

E content of 20 grams cassava + 4 grams rice bran + 0, 4 gram $MgSO_4$ + 1 gram $CaCO_3$.

F content of 20 grams cassava + 4 grams rice bran + 0,5 gram $MgSO_4$ + 1 gram $CaCO_3$.

Distribute each treatment into heat-resistant nylon bag. Then cover the steamed cotton button in 121^0C, 1 atm, for 30 minutes.

Then let cool and implant mycelium Ganoderma lucidum. The mycelium begins to grows in temperature 24^0C.

III. Result and Discussion

A. *Mycelium Growth of Ganoderma lucidum in PDA (Potato Dextrose Agar)*

As shown in Fig. 1 this is the first step to creating G.lucidum.

After one day of isolation, the mycelium of Ganoderma lucidum began to adapt to the PDA on 1 day, then the mycelium cling to PDA (Potato Dextrose agar) and continue to cling to the mycelium from the inside out for up from 1 day to 10 days Fig. 2 at 24^0C at during day and night, the mycelium full test tube, fine mycelium.

1 day 3 days 6 days 9 days 10 days

Figure 2. Mycelium growth of Ganodera lucidum in PDA

G.lucidum mycelium Spread quickly and strongly to ensure seed sources for growing mushrooms.

B. *Mycelium Growth of Ganoderma lucidum in Brown Rice*

After 2 days of isolation, Ganoderma lucidum mycelium begins to grow long mycelium. After 7 days, the G.lucidum mycelium spinning mycelium and deep into brown rice. Mycelium white, forming a cotton-like layer surrounding the nutrient medium. Mycelium core wool surrounds each grain of brown rice.

After 10 days, the G. lucidum mycelium starts to slowly turn yellow due to the nutrients of the brown rice. Ganodrema lucidum mycelium is absorbed by the mosquito.

G.lucidum mycelium 2 days | G.lucidum mycelium 7 days | G.lucidum mycelium 10 days

Figure 3. Mycelium growth of Ganodera lucidum in brown rice

As shown in Fig. 1 and Fig. 3, this is the first step to creating pure, G.lucidum mycelium spread quickly and strongly to ensure seed sources for growing mushrooms.

C. *Ganoderma lucidum Mycelium Gorwth Grain Mix Rice Bran and MgSO4*

Speed of Ganoderma lucidum mycelium increased from 2 to 8 days. During 2 days, Ganoderma lucidum mycelium slows down as Ganoderma lucidum mycelium begins to adapt to the new nutrient composition, but on day 4 and day 6 the Ganoderma lucidum began to adapt and the speed of mycelium spread

On the 4 days, Ganoderma lucidum mycelium R0 (1,7 ± 0,1b) cm, R1 (1,3 ± 0,1a) cm, R2 (1,6 ± 0,1b)cm, R3 (1,4 ±0,21a)cm, R4 (1,3 ± 0,1a)cm. The 4 days, Ganoderma lucidum mycelium growth in R2 (1,6 ± 0,1b) cm treatment was faster than that of other treatments, healthy, white, uniform fungal filament, nutrient uptake 100 g grain supplemented with 0,2 g $MgSO_4$ and 1 g $CaCO_3$ and 5 g rice bran more than R0, R1, R3, R4

On the 6 days, Ganoderma lucidum mycelium R0 (3,2 ± 0,3b)cm, R1 (2,6 ± 0,2a)cm, R2 (3,0 ± 0,2 b)cm, R3 (2,6 ± 0,2a)cm, R4 (2,4 ± 0,2a)cm. The 6 days, Ganoderma lucidum mycelium growth in R0 (3,2 ± 0,3b) cm treatment was faster than that of other treatments, healthy, white, uniform fungal filament, nutrient uptake 100 g grain supplemented with 1g $CaCO_3$ and 5g rice bran more than R1, R2, R3, R4

On the 8 days, Ganoderma lucidum mycelium has spread the bottle. Treat R0, R1, R2, R3, R4 with white mycelium, Ganoderma lucidum mycelium in R0 treatment at day 8 (5,9 ± 0,2c) cm, R 1 (4,8 ± 0,3a)cm, R2 (5,6 ± 0,5b)cm, R3 (4,8 ± 0,2a)cm, R4 (4,7 ± 0,2a)cm. The 8 days, Ganoderma lucidum mycelium growth in R0 (5,9 ± 0,2c) cm treatment was faster than that of other treatments, healthy, white, uniform fungal filament, nutrient uptake 100 g grain supplemented and 1 g $CaCO_3$ and 5 g rice bran more than R1, R2, R3, R4. The R0 is optimal.

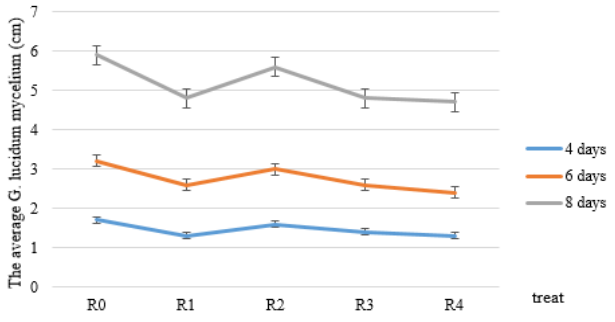

Figure 4. Mycelium growth of Ganoderma lucidum in grain mix rice bran and $MgSO_4$

Figure 5. Ganoderma lucidum mycelium 2 days on grain

Figure 6. Ganoderma lucidum mycelium 4 days on grain

Figure 7. Ganoderma lucidum mycelium 6 days on grain

Figure 8. Ganoderma lucidum mycelium 8 days on grain

As shown in Fig. 4 and Fig. 5, Fig. 6, Fig. 7, Fig. 8, Table I is in addition to the PDA.

G.lucidum mycelium spread quickly, strong, healthy, pure breed, brown rice is a replacement component of PDA to ensure the source of seed for mushroom cultivation needs.

TABLE I. THE MYCELIUM GROWTH GANODERMA LUCIDUM (CM) ON THE GRAIN MIX $MgSO_4$ AND RICE BRAN

Treatment	The mycelium growth Ganoderma lucidum (cm) on the grain mix $MgSO_4$ and rice bran, $CaCO_3$.		
	4 days	6 days	8days
R0	1,7 ±0,1b	3,2 ±0,3b	5,9 ±0,2c
R1	1,3 ± 0,1a	2,6 ± 0,2a	4,8 ± 0,3a
R2	1,6 ± 0,1b	3,0 ± 0,2 b	5,6 ± 0,5b
R3	1,4 ±0,21a	2,6 ± 0,2a	4,8 ± 0,2a
R4	1,3 ± 0,1a	2,4 ± 0,2a	4,7 ± 0,2a

(a, b, c,… show statistical difference, p -value <0,05)

D. *Mycelium Gorwth of Ganoderma lucidum in Vasscara Substract Treated Supplemented with $MgSO_4$ with Different Ratios and 4 g Rice Bran and 1 g $CaCO_3$*

According to the Fig. 9, Fig. 10, three days mycelium Ganoderma lucidum in the treatments according to Table II was attached to and spread into cassava substrate with

added $MgSO_4$ and 4 g rice bran and 1 g $CaCO_3$, A (1,7 ± 0,2b) cm, B (2,0 ± 0,3b) cm, C (2,2 ± 0,2b) cm, D (1,8 ± 0,2b)cm, E (2,1 ± 0,2b)cm, F (1,9 ± 0,2b)cm.

Fig. 11, 6 days Ganoderma lucidum mycelium treatment B (7,4 ± 0,5d) cm is stronger than other treatment A (5,8 ± 0,3c) cm, C (7,2 ± 0,2d) cm, D (7,1 ± 0,4d)cm, E (5,7 ± 0,1c)cm, F (5,73 ± 0,07c)cm.

Fig. 12, 9 days Ganoderma lucidum mycelium treatment B (11,1 ± 1,9fg) cm is stronger than other treatment A (10,5 ± 0,4ef), C(10,45 ± 0,05ef), D(10,2 ± 0,2e), E(9,8 ± 0,5e), F(10,38 ± 0,58ef).

Fig. 13, 12 days mycelium in treatment A reached (12,1 ± 0,8hi cm), mycelium in treatment B reached (14 ± 0,3j cm), mycelium in treatment C reached (11,56 ± 0,06 ghi cm), mycelium in treatment D reached (11,35 ± 0,15g cm), mycelium treatment E reached (11,4 ± 0,2gh cm), mycelium in treatment F reached (12,3 ± 0,4i cm).

The mycelium of Ganoderma lucidum in treatment B began to spread faster than mycelium in the remaining treatments, mycelium in treatment B on day 6 reached (7,4 ± 0,5d cm), day 9 reached (11,1 ± 1,9fg cm), day 12 (14 ± 0,3j cm)

Therefore, vasscara substrate has been processed MgSO4 0,1 (g) in B treatment was suitable for rapid spread of white mycelium.

As shown in Fig. 9, Table II shows that vassava waste in agriculture is used as a substrate for nourishment Mushroom fungus is very suitable. This is vassava easy to find Vietnam, cheap price, easy to find to apply to raising silk fungus.

TABLE II. THE MYCELIUM GROWTH GANODERMA LUCIDUM (CM) ON THE BASE VASSAVA SUBSTRATE TREATED SUPPLEMENTED WITH $MgSO_4$ DIFFERENT RATIOS AND 4 GRAMS RICE BRAN AND 1 GRAM $CaCO_3$

Treatment	The mycelium growth Ganoderma lucidum (cm) on the base vassara substrate treated supplemented with $MgSO_4$ different ratios and 4 g rice bran and 1 g $CaCO_3$			
	3 days	6 days	9 days	12 days
A	1,7 ±0,2b	5,8 ±0,3c	10,5 ±0,4ef	12,1 ±0,8hi
B	2,0 ±0,3b	7,4 ±0,5d	11,1±1,9 fg	14 ±0,3j
C	2,2 ±0,2b	7,2 ±0,2d	10,45 ±0,05ef	11,56 ±0,06 ghi
D	1,8 ±0,2b	7,1 ±0,4d	10,2 ±0,2e	11,35 ±0,15g
E	2,1 ±0,2b	5,7 ±0,1c	9,8 ±0,5e	11,4 ±0,2gh
F	1,9 ±0,2b	5,73 ±0,07c	10,38 ±0,58ef	12,3 ±0,4i

(a, b, c,… show statistical difference, p -value <0,05)

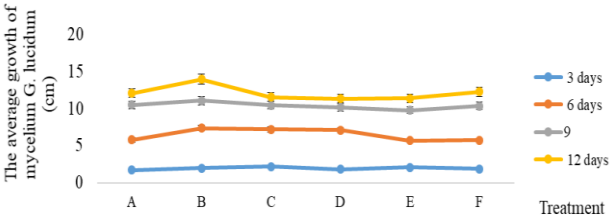

Figure 9. Graph the average growth of mycelium G.lucidum on the base vassara substrate treated supplemented with $MgSO_4$ different ratios and 4 g rice bran and 1 g $CaCO_3$

Figure 10. Mycelium gorwth of Ganoderma lucidum in vasscara substract treated supplemented with MgSO4 with different ratios and 4 (g) rice bran and 1 (g) $CaCO_3$ 3 days

Figure 11. Mycelium gorwth of Ganoderma lucidum in vasscara substract treated supplemented with MgSO4 with different ratios and 4 (g) rice bran and 1 (g) $CaCO_3$ 6 days

Figure 12. Mycelium gorwth of Ganoderma lucidum in vasscara substract treated supplemented with MgSO4 with different ratios and 4 (g) rice bran and 1 (g) $CaCO_3$ 9 days

Figure 13. Mycelium gorwth of Ganoderma lucidum in vasscara substract treated supplemented with MgSO4 with different ratios and 4 (g) rice bran and 1 (g) $CaCO_3$ 12 days

E. Data Processing

Statgraphics centurion XV

IV. CONCLUSION

Isolation of Ganoderma lucidum mycelium on PDA (Potato Dextrose agar) and Isolation Created Ganoderma lucidum Mycelium on Brown Rice. G.lucidum mycelium spread quickly, strong, healthy, pure breed, brown rice is a replacement component of PDA to ensure the source of seed for mushroom cultivation needs.

Effect of $MgSO_4$ on Growth G.lucidum Mycelium with Supplementation of 5 (g) Rice Bran on Grain Therefore, vasscara substrate has been processed $MgSO_4$ 0,1 (g) in B treatment was suitable for rapid spread of white mycelium.

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Nguyen Thi Tuyet Nhung was born in 1979, Master, lecture in biotechnology Ho Chi Minh City University of Food Industry, Viet Nam.

She completed her master's degree in 2012 at Ho Chi Minh City University of education in Vietnam. In 2014, she officially became a lecturer in the biotechnology department of the Ho Chi Minh City University of Food Industry (HUFI), Vietnam.

Her interests include Isolation and Mycelium Growth of Ganoderma lucidum on Manihot Esculent Substrate with Mineral Supplement.