# Isolation and Production of Polysaccharide from Locally Isolated *Termitomyces* sp. Mushroom

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***Abstract.*** *Termitomyces* sp. mushroom is an edible mushroom that belongs to the family of Lyophyllaceae and exhibited growth associated with termites in a symbiotic environment. In this study, Termitomyces sp. mushroom was isolated from several places located in Negeri Sembilan, Malaysia namely TM 1 (Senaling, Kuala Pilah, TM 2 (Batu Kikir, Kuala Pilah) and TM 3 (Rembau Negeri Sembilan). The collection process was conducted during the early rainy seasons within October to November in which these mushrooms were actively growing. The collected mushrooms fruiting bodies from the wild were further used in tissue culture for the growth of mycelium on the agar medium. Out of the three samples only TM 1 mycelium were successfully grown on the agar medium and subsequent optimization were carried out to enhance the growth of the TM 1 mycelium. The composition of the agar medium was manipulated, and it was observed that the combination of PDA (potato dextrose agar) + ME (malt extract) + YE (yeast extract) showed a thick full plate growth of mycelium in 14 days of cultivation. The mushroom mycelium of TM 1 was able to grow and produce polysaccharide in batch submerged liquid fermentation with a biomass of 8.55 g/L, intracellular polysaccharide (IPS CWE and IPS HWE) of 1.36 and 3.20 g/L respectively and extracellular polysaccharide of 1.44 g/L.
***Keywords*****:** Termitomyces; mushroom; polysaccharide; submerged fermentation.

## INTRODUCTION

In the normal human diet edible mushrooms have become integral part and received more attention based on their safety and considered as functional food or nutraceutical product [5]. In addition to that they are considered as healthy food because their mineral content is higher than that of meat or fish and most vegetables, apart from their nutritional value mushrooms have potential medicinal benefits because, many mushrooms reported to produce a wide range of secondary metabolites having high therapeutic values such as antioxidant, antitumor, antibacterial, antiviral, and immunomodulating properties [3].

*Termitomyces* sp. is a genus of edible mushrooms commonly consumed in Africa and Asia among the mushrooms collected from the wild [4]. Apart from protein, *Termitomyces* sp. is an affluent source of sugar, fibre, lipid, vitamin, mineral in addition to medicinal value which is used in lower blood pressure, rheumatism, kwashiorkor, obesity, diarr [6]. *Termitomyces* sp. is a popular wild edible mushroom grown in termite gut where they produce various enzymes to help termites digest lignocellulosic substrates [8]. In Negeri Sembilan, Malaysia, this mushroom is normally consumed as local food and believed by the local people that it benefits to their health. To date there is no scientific prove reported on the ethnomedicinal properties and the cultivation technique of *Termitomyces* sp. mushroom mycelium.

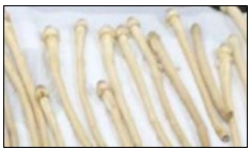
In this research the collected mushroom from Negeri Sembilan was grew through tissue culture technique and the composition of the agar medium was manipulated to optimize a suitable growth medium for *Termitomyces* sp. mushroom.

## METHOD AND MATERIALS

*A. Mushroom Collection*

The fruiting bodies of *Termitomyces* sp. was successfully obtained from Kampung Sungkak, (Senaling), Batu Kikir and Rembau which are located in Negeri Sembilan. Table I shows the coordinates and the image of obtained *Termitomyces* sp. sample.

TABLE I
COORDINATES AND THE IMAGE OF THE OBTAINED *TERMITOMYCES* SP. MUSHROOM SAMPLE

| Area | Code | Picture |
|---|---|---|
| Senaling, Kuala Pilah<br>Latitude 2.7069° N<br>Longitude 102.2484° E | TM 1 |  |
| Batu Kikir, Kuala Pilah Latitude 2.8338° N<br>Longitude 102.3147° E | TM 2 |  |
| Rembau, N. Sembilan Latitude 2.5905° N<br>Longitude 102.0930° E | TM 3 |  |

*Agar Medium Preparation (PDA, PDA + YE, PDA + ME, PDA + YE + ME)*

The mixture was dissolved and subsequently autoclaved at 121°C for 15 minutes at 15 psi. Once it is cooled down, it was poured into petri plates and left to harden. The plates were stored at 4°C in refrigerator for further usage. Manipulation of agar medium was carried out by changing the composition of the medium as shown in table II.

TABLE II
TYPE OF MEDIUM AND AMOUNT OF PDA, YEAST EXTRACT AND MALT EXTRACT ADDED TO THE COMPOSITION

| Medium | Composition | Amount (g/L) |
|---|---|---|
| Medium 1 | PDA | PDA = 39 |
| Medium 2 | PDA + YE | PDA = 39, YE = 4 |
| Medium 3 | PDA + ME | PDA = 39, ME = 4 |
| Medium 4 | PDA + YE + ME | PDA = 39, YE = 4, ME = 4 |

*PDA= Potato Dextrose Agar, YE = Yeast Extract, ME = Malt Extract

*Production of Polysaccharide*

1. *Submerged Liquid Fermentation (SLF)*: Submerged liquid fermentation was carried out for the production of mycelium. A 5 mm plug of *Termitomyces* sp. mycelia was removed with a cork borer from 10 day-old-culture of agar medium. The mycelia cut (10 plugs) was transferred into liquid medium incubated in orbital shaker for 7 days at 28°C, 170 rpm for mycelia growth. Cultured mycelia were then dried using oven (50°C) until constant weight. Dried mycelia were used for intracellular polysaccharide (IPS) extraction using ethanol, cold water and hot water method. Culture filtrates were used for extracellular polysaccharide (EPS) extraction using ethanol [9].

*Extraction of Polysaccharides*

*1) Cold Water Extraction, CWE*: Approximately, 5 g of mycelia powder were mixed with 250 mL of distilled water and stirred vigorously for 3 hrs at room temperature. The extracts were filtered using Whatman No 1 and the culture filtrates were dried in the oven at 35° for intracellular polysaccharides cold water extracts (IPS CWE). Exopolysaccharide cold water extract were prepared by directly drying the culture filtrate (after separated from mycelium) in the oven at 50°C; yielding EPS CWE [2].

*2) Hot Water Extraction, HWE*: Intracellular polysaccharide hot water extracts (IPS HWE) were extracted. Briefly, 20 g of dried mycelia powder was extracted twice with distilled water (250 mL) at 80°C for 3 hrs in water bath. The extracts were cooled, filtered and the filtrates were dried at 50°C in oven [2].

3) *Ethanol Extraction, EE*: Culture filtrates were mixed with 4 times of absolute ethanol (1:4 ratio), stirred vigorously and kept overnight at 4°C. The mixture was filtered using Whatmann No.1 filter paper and polysaccharide were dried at 50°C in the oven yielding extracellular polysaccharide ethanol extracts (EPS EE). For intracellular polysaccharide ethanol extracts (IPS EE), a modified hot water extraction used. Briefly, the powders of dried mycelia (20 g) were extracted twice with distilled water (250 mL) at 80°C for 3 hrs in a water bath. The extracts were cooled, filtered using filter paper (Whatmann No. 1) and precipitated using ethanol (1:4) before drying in the oven at 30°C [1].

## RESULTS AND DISCUSSION

### *A. Growth of Mycelium on the Agar Medium After Collecting All the Sample*

Out of the three samples collected only TM 1 was able to grow on PDA subsequently after collected from the sampling site. However, TM 2 and TM 3 were not able to grow on PDA. Thus, growth of TM 1 mycelium was further enhanced by manipulating the agar medium composition. Table III shows the diameter of TM 1 mycelium in day 9, 11, 14 and 16 cultivation period with different composition of agar medium. For PDA medium the highest diameter achieved was 5 mm from day 9 to day 11. The combination of PDA + YE showed highest diameter of 29 mm at day 16 whereas for PDA + ME the diameter of mycelium increased to 20 mm (day 9) to 29 mm (day 16). It was observed that TM 1 had the greatest growth on the combination of PDA + YE + ME without any sign of contamination and the diameter of mycelium increased from 29 mm (day 9) to 33 mm (day 11) and reached full plate (45 mm) in day 14 and day 16. Table 3 shows the diameter of TM 1 mycelium on different cultivation period and agar composition.

TABLE III
DIAMETER OF *TERMITOMYCES* (TM 1) MYCELIUM WITH DIFFERENT CULTIVATION PERIOD AND AGAR MEDIUM COMPOSITION

| Days | Agar Composition | Diameter (mm) |
|---|---|---|
| 9 | PDA | 5 |
| | PDA + YE | 17 |
| | PDA + ME | 20 |
| | PDA + YE + ME | 29 |
| 11 | PDA | 5 |
| | PDA + YE | 19 |
| | PDA + ME | 25 |
| | PDA + YE + ME | 33 |
| 14 | PDA | 5 |
| | PDA + YE | 22 |
| | PDA + ME | 30 |
| | PDA + YE + ME | FP |
| 16 | PDA | 5 |
| | PDA + YE | 29 |
| | PDA + ME | FP |
| | PDA + YE + ME | FP |

*FP = Full plate

*B. Mycelium Biomass Production*

Submerged liquid fermentation (SLF) was further carried out to determine the ability of isolated mycelium (TM 1) for growing and producing polysaccharide in liquid medium. Results show that TM 1 mycelium able to grow in SLF and produce intracellular polysaccharide (IPS) and extracellular polysaccharide (EPS). After 7 days of incubation in SLF, the mycelium biomass obtained was 8.55 g/L. The dried mycelium was further extracted in hot and cold water for IPS which were 1.36 g/L and 3.20 g/L IPS for CWE and HWE respectively. The EPS was obtained from the supernatant of liquid fermentation medium and the yield obtained was 1.44 g/L.

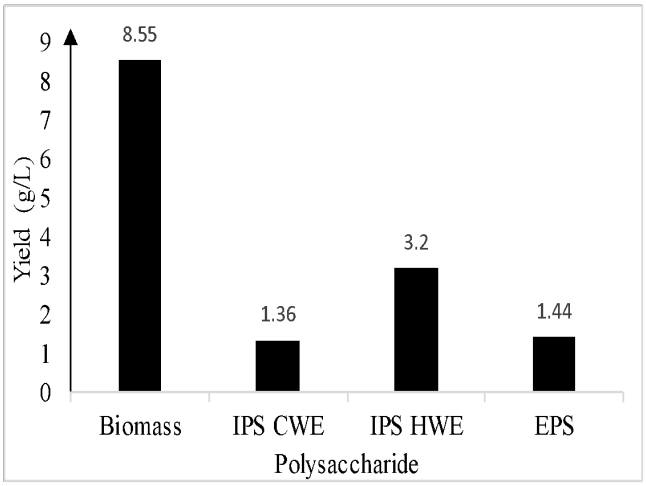



**Fig. 1** Yield of polysaccharide obtained from SLF of TM 1 mycelium

As shown in figure 2 below, TM 1 mycelium pellet in 7 days cultivation. The pellet is small and dense. The dense pellet of mycelium indicates high concentration of IPS. It was stated that the round and small pellet observed in this experiment allows a high concentration of EPS due to the higher surface area of the mycelium pellet. This result was similar with previous research which reported that the EPS were highly secreted from the round and small size of pellet mycelium [7].

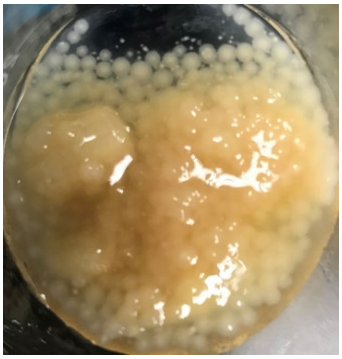


**Fig. 2** Mycelium pellet formed from submerged liquid fermentation

## CONCLUSIONS

In conclusion, the agar medium composition of PDA + YE + ME is the best combination to grow *Termitomyces* sp. mycelium (TM 1) as it showed full plate growth without any sign of contamination. *Termitomyces* sp. was successfully grew through submerged liquid fermentation thus, the polysaccharide of this seasonal mushroom could be produced at any time through submerged liquid fermentation without depending on the seasons.

## NOMENCLATURE

| | | |
|---|---|---|
| a | milliliter | mL |
| b | gram per liter | g/L |
| c | degree Celsius | °C |
| d | grams | g |


## ACKNOWLEDGEMENT

We would like to thank Causal Productions for permits to use and revise the template provided by Causal Productions. Original version of this template was provided by courtesy of Causal Productions (www.causalproductions.com).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