Alternative substrate for *Hypsizygus tessellatus* cultivation

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI, MALAYSIA

April 2017

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ABSTRACT

The demand of mushroom is increasing annually which open a huge opportunity to developing countries to expand their mushroom industries as the mushroom culturing process converts agriculture waste to profit from crops. *Hypsizygus tessellatus* is a popular worldwide cultivated edible mushroom which is high in nutrient and medicinal properties. This study is conducted to determine the effect of different parts of the stalk and different strength of potato dextrose agar (PDA) on the mycelium growth of *H. tessellatus* and to determine the effect of different alternative substrate, namely *Dicranopteris linearis* (fern leaf), dry leaf of *Terminalia catappa* and *Imperata cylindrica* (lalang grass) on the growth of shimeji mushroom, *H. tessellatus*. The mycelium from the upper and lower part of the mushroom stalk was cultured on half strength and full strength PDA, and the radius of the mycelium growth were measured. Mycelium from the lower part of the stalk have better growth effect. Full strength PDA also shown significantly better growth effect in mycelium growth. *H. tessellatus* were cultured on different types of substrate in capsule. Small pieces of *D. linearis*, *I. cylindrica* and dried leaf of *T. catappa* were found suitable to be used as alternative substrate for *H. tessellatus* cultivation where the mycelium growth in these substrate have no significant difference from the commercially used substrate which is mixture of saw dust and rice bran. The three alternative substrate mentioned above can be found in urban area in large amount which gave opportunity to people couldn't gain access to the commercial substrate chance to collect their own substrate for mushroom cultivation.

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LIST OF ABBREVIATIONS

mt/ha	metric ton/hectare
°C	degrees Celsius
pH	potential of hydrogen
g	gram
g kg^{-1}	gram per kilogram
%	percent
mm	millimeter
cm	centimeter
mL	milliliter
kPa	kilopascal
PDA	Potato dextrose agar
C: N ratio	carbon-to-nitrogen ratio
C	contról, commercial substrate
FA	small pieces of *D. linearis*
FB	powder form of *D. linearis*
LA	small pieces *I. cylindrical*
LB	powder form *I. cylindrical*
DA	small pieces *T. catappa*'s dried leaf
DB	powder form *T. catappa*'s dried leaf

1.0 CHAPTER 1

INTRODUCTION

Mushroom is one of the most well known fungi which is categorize in the class of Basidiomycetes and can be easily found growing on damp rotten wood trunk of trees in the forest or moist soil rich in organic substance (Rajapakse, Rubasingha & Dissanayake, 2007). Unlike plants that convert solar energy to chemical energy, mushroom like any other fungi, which does not contain chlorophyll utilize organic matters from plant, animal or other fungi as nutrition and energy source (Nasim et al., 2001). Edible mushroom can be easily found on the shelf of food market nowadays and are also popular in the list of the food menu as people love its special texture, flavour and aroma. It can be cooked as main ingredient or used as condiment for other dishes. Mushrooms are known to have high value of proteins, carbohydrates, multi vitamins, minerals, rich in folic acid (vitamin B) (Mat Amin, Azahar Harun, & Abdul Wahab, 2014), and most of the essential amino acid (Mujić, Zeković, Lepojević, Vidović, & Živković, 2010). Mushroom could also prevent hypertension, high cholesterol and cancer (Mujić, Zeković et al., 2010) which increases its demand due to consumers having the awareness that consuming mushrooms are benefit to health. The Malaysia's National Argo-Food Policy (2011- 2020) has also listed mushroom as one of the high-value commodities due to its potential and promising high market demand. According to Mat Amin et al. (2014), the demand for mushrooms in the world is increasing and this will bring the opportunity for expanding the Malaysia mushroom industry as the climate of Malaysia is suitable for the cultivation of 17 varieties of edible mushroom.

According to Sanchez (2004), cultivation of edible mushroom is a biotechnology process, which aids in reducing and equally protecting the environment from excess solid waste. This has been an attractive point for developing countries to involve themselves in culturing mushrooms which converts agriculture waste to profit enable crops (Khan et al., 2012). Cultivation of mushroom using cheap substrate has also been studied in recent years as the process could convert waste product to nutritious food or therapeutic compounds (Tetiana & Victor, 2015). Many studies have reported the suitability of various substrates for mushroom cultivation including rice straw, maize, oak wood, horse

chest nut, saw dust, cotton stalk and others (Zervakis, Philippoussis, Ioannidou, & Diamantopoulou, 2001). In Malaysia, sawdust and rice husk are commonly used for fungal cultivation (Saidu, Salim, & Yuzir, 2011). However, most of these substrates are not available in urban areas compared to other housing wastes such as wild grass or dried leaf. By finding new alternative substrate, we could lower the cost in mushroom production which will bring benefit and provide opportunity for urban area people to start growing mushroom for small scale business or self-sufficiency for food production. By further studying and development of cultivation technique, people could also can start to grow their own mushroom at home as the substrate could be easily found around and can be collected in large and sufficient amounts such as fern leaf, grass or dried leaf.

Hypsizygus tessellatus commonly known as Buna shimeji mushroom is a commonly cultivate mushroom in East Asian. It contain various kind of vitamins, polysaccharides, and amino acids which has high medicinal value such as antitumor, antiaging, controlling cholesterol, weight loss and constipation (Khondkar et al., 2012). Beside its medicinal value, *H. tessellatus* also been as a food source in the Eastern or Western countries. It said to have a crunchy texture and a sweet taste just like crab meat when it is cooked (Waites et al., 2001). Therefore *H. tessellatus* is a valuable mushroom species which has a market demand and potential to be widely cultivate by reducing its cultivation cost and increasing the harvesting yield.

There were many studies in Malaysia carried out using *Pleurotus ostreatus*, oyster mushroom, but not many studies carried out on *H. tessellatus*. To date, study related to the effect on *H. tessellatus* growth of different parts of the stalk and different strength of potato dextrose agar (PDA) were not focus. If different strength of PDA gave the same effect for the mycelium growth, the half strength PDA can be used instead of the full strength PDA which will be more cost effective. Substrates such as Dicranopteris *linearis* (fern leaf), dry leaf of *Terminalia catappa* and *Imperata cylindrica* (lalang grass) have not been studied as an alternative substrate for the culturing of *H. tessellatus*. Therefore, the aim of this study is to determine the effect of different parts of the stalk and different strength of PDA on the mycelium growth of *H. tessellatus* and to determine the effect

of *D. linearis*, dry leaf of *T. catappa* and *I. cylindrica* as an alternative substrate for the culturing of shimeji mushroom, *H. tessellatus*.

2.0 CHAPTER 2

LITERATURE REVIEW

2.1 MUSHROOM INDUSTRY

Between 1990 and 2012, the main producers of mushroom in the world were China, USA, Poland, Spain and Netherlands, and the top producer among these countries was China. Mushrooms are traded in the world in fresh, dried, canned and processed mushroom based products (Mat Amin, Azahar Harun, & Abdul Wahab, 2014). The demand of mushroom in Malaysia is expected to increase from 20000 tons in 2008 to 48000 tons in 2020. The demand of mushroom in the global market is also high, therefore it is a great opportunity for Malaysia to grow the mushroom industry. The Malaysian government has decided to expand the mushroom farms from 78 hectares in 2010 to 340 hectares in 2020 (Mat Amin, Azahar Harun, & Abdul Wahab, 2014). The Malaysia National Agro-Food Policy (2011-2020) also planned to increase the total productivity from 190 mt/ha (2010) to 193 mt/ha (2020) by formulating strategy to strengthen the mushroom industries. The strategies for strengthen the mushroom industries in Malaysia is by improving the production system of mushroom, expansion of land to increase the productivity and to set up a mushroom bag centre to distribute the mushroom bag to the farmer to ensure the quality of each batch of culture (Mat Amin, Azahar Harun, & Abdul Wahab, 2014).

2.1.1 *Hypsizygus tessellatus*

In the division Thallophyta, mushroom were classified as lower plant by Linnaeus due to its simple structure lacking of true roots, true leaves, true flower, true steam, and true seeds (Chang & Miles, 2004). To identify the mushroom, basic knowledge about the macroscopic structure is required. Mushroom that appear on market are edible mushroom which are defined to be absence of poisonous effects on humans with desirable taste and aroma (Mattila, Suonpää, & Piironen, 2000). *H. tessellatus* also called *H. marmoreus* which is class under the Tricholomataceae, Agaricales, Hymenomycetidae, Basidiomycetes, is a popular worldwide cultivated edible mushroom native to East Asia

mainly in Japan (Imtiaj et. al., 2016). The mushroom is often found growing on beech tree in bunch with 15- 50 individuals like small light brown or white umbrella which commonly called shimeji (pleated umbrella in Japanese) or beech mushroom. The mushroom are advised to be cooked since it give a bitter taste when eaten raw. The bitter taste will eventually disappear upon cooking and the mushroom is claimed to have the taste of crab meat with a crunchy texture and delicate mild, sweet flavor (Waites et al., 2001).

According to Akavia et al., (2009), the *H. tessellatus* mycelium has the best growth rate at temperatures of 20–25°C, media pH of 7 in potato dextrose agar (PDA) and yeast–malt extract. The most suitable carbon and nitrogen source for the mycelium growth are maltose and calcium nitrate, $CaNO_3$.

H. tessellatus contain various kinds of polysaccharide and eight sets of necessary amino acids (Akavia et al., 2009), which most of them have high potential or have been proven to have important medicinal purpose. Studies have been done which claim that consuming *H. tessellatus* could inhibit tumor growth (Ikekawa et al., 1992; Kaneko et al., 2015), inhibit growth of leukaemic cells (Tsai, Ma, & Wu, 2013), treat platelet related cardiovascular disease, including thrombosis, stroke and atherosclerosis and has potential antimicrobial effect with moderate anti-oxidant effect (Ji Young et al., 2011).

2.2 SUBSTRATE FOR CULTIVATION OF *H. tessellatus*

There are many kinds of substrate that can be used for the cultivation of *H. tessellatus*. A suitable substrate should be biodegradable and contain nutrient that support mycelium growth. Different substrates may have different effect to the mycelium growth.

2.2.1 Saw dust

Saw dust or the wood dust are easily found in the wood factory as a byproduct. They are produced when wood or log were drilled, cut, sanded or ground. Saw dust can be used as mulches to improve growth of crops, improve structure and aeration of heavy soils, increase water infiltration, reduce surface runoff and improve aggregation of surface soil (Roberts & Mellenthin, 1959). The estimated nutrient of saw dust is 1.2 g kg^{-1} nitrogen,

7.2 g kg^{-1} phosphorus, 7.2 g kg^{-1} potassium, 23.6 g kg^{-1} calcium, 5.2 g kg^{-1} magnesium and carbon: nitrogen ratio of 189.2 g kg^{-1} (Odedina, Ojeniyi, & Awodun, 2007).

2.2.2 Rice bran

Rice bran is the hard outer layer of the fresh harvested rice is produced as a byproduct of milling during the conversion of brown rice to white rice. It contain various antioxidant that are beneficial to human health (Barron, 2010). The bran contain the aleurone of the rice which is rich in protein of maturing seeds. According to NutrientData.com (2014), 118 g of rice bran contains 25 g of fat, 6 mg of sodium, 59 g of carbohydrate and 16 g of protein. The rice bran is usually used as an ingredient in bakery and breakfast cereals as it contains a high value of dietary fiber. Rice brain is also one of the main ingredients used for mushroom cultivation in Malaysia (Saidu, Salim, & Yuzir, 2011).

2.2.3 Dried leaf (*Terminalia catappa*)

Leaves fall every day from trees and dry up on the ground, but people are not aware that leaves have uses and is nutritious. Studies have shown that dried leaves in general contain 52% of carbon, 1% of nitrogen, 0.23% of phosphorus, 1.64% of calcium, 0.24% of magnesium and 0.11% of sulphur (Joseph, Daniel, & Donn, 1988). Dried leaves are usually used as mulch to lessen the growth of weeds, retain soil moisture, maintain lower soil temperatures in the summer and improve soil structure (Subramanian, 2012). Mushrooms are naturally found growing on dead wood, therefore dried leaves which may have similar nutrient value with dried twigs that are part of the dead wood may become a potential substrate that could be used as an alternative substrate for mushroom cultivation.

T. catappa commonly known as Indian Almond, Malabar Almond, and Tropical Almond (Nadkarni, 1976), is mostly planted as an ornamental, shade, and salt-tolerant street tree (Chanda, Rakholiya, & Nair, 2011). The tree has a large leaf, 15- 25 cm long and 10- 14 cm broad, which falls every day covering the land. The fallen leaf turns from red to yellow and finally becomes a brown dried leaf. The leaf could serve as Tasar silkworm food. By littering a few dried leaf of *T. catappa* in the aquarium, tannins will be released to lower the pH of water and exhibit antifungal and antibacterial properties (Mari, 2014). Besides, the leaf also contain several medicinal properties such as anti-

inflammatory (Lin, Hsu, & Lin, 1999), anti-hepatitis (Chen, Li, Liu, & Lin, 2000), anti-HIV reverse transcriptase (Tan, Pezzuto, Kinghorn, & Hughes, 1991), anticancer, antioxidant (Masuda et al., 1999), therapeutic effects for liver related diseases (Chiu, & Chang, 1986), headache and colic (Morton, 1985). Although the dried leaves if *T. catappa* has huge medicinal potential, but it was still not been widely used in the field. The dried leaves fall every day which could be easily collected in large amount to serves as substrate for mushroom cultivation.

2.2.4 Straw of *Imperata cylindrical*

Straw are usually referred as an agricultural byproduct from dried stalks of cereal plant such as barley, oat, rice and wheat. *I. cylindrical* commonly known as the cogon grass or lalang grass locally is a type of straw that can be easily found in any grassy land or field. These straw are known to be rich in lignin and hemicellulose which are important for the plant structural support (Martone et al., 2009). Both of the lignin and hemicellulose are hydrocarbons that will serves as a food source for the mycelium growth. In addition, the lalang grass is also used in traditional Chinese medicine and has cooling properties which is good for lungs, stomach and urinary bladder channels ("Imperata (bai mao gen)", 2009).

2.2.5 *Dicranopteris linearis*

D. linearis also named *D. dichotoma* is a perennial fern of the family Gleicheniaceae (Wang et al. 2003), which grows in humid subtropical and tropical regions (Xu et al., 2014). The fern can be easily found growing at roadsides, hills, forest floors and abandoned property. It plays a role as early-stage colonizer (Zhao et al., 2012), growing on acidic or oligotrophic soil and having high tolerance to nutrient lacking environment (Wang et al., 2003). The growth of the fern creates a dense layer on the forest floor which forms a favorable soil microclimates that could increase the soil biota and ecological processes (Liu, Wu, Zhou, Lin, & Fu, 2012). The leaf of *D. linearis* takes a long time to be decomposed and forms a thick network of dead fern which could reach a meter thick. The fern rhizomes and roots cold penetrate through the network and use the network layer as its own substrate (Russell, Raich, & Vitousek, 1998).