Optimal mycelial conditions and spawn production for the domestication of *Macrolepiota deters*

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Abstract

Macrolepiota detersa is an edible saprobic basidiomycota, known to grow solitary to scattered on decayed litter layers. This mushroom produces a relatively large striking basidiomata containing light brown detachable squamules on the white pileal background. In this study, *M. detersa* MFLUCC 13-0901 and SIMA 13266, isolated from Chiang Rai, Thailand was investigated for optimal conditions for mycelial growth and spawn production. Among nine cultural media tested, MEA (malt extract agar) was the best medium. This fungus was able to grow at a wide ranges of temperature and pH values. The optimal temperature and pH for the mycelial growth were at 30 ºC and pH 7.0 or 8.0 respectively. Among nine different selected substrates for spawn preparation, barley and red sorghum were the best media. Spawn were obtained after 14 days following inoculation.

Keywords: *Macrolepiota detersa*, mycelial growth, spawn production

Introduction

Macrolepiota detersa is a white spored, gilled mushroom of the genus *Macrolepiota* (Agaricaceae, Agaricales, Basidiomycota) identified in 2010 (Ge et al., 2010). This mushroom is known to be an edible species. It produces a relatively large striking basidiomata containing light brown detachable squamules on the white pileal background. *Macrolepiota detersa* resembles with *M. procera* but it can be easily separated from *M. dolichaula*. *Macrolepiota detersa* contains larger plate like squamules which can be easily detached as compared to *M. dolichaula*. It is a terrestrial saprotrophic forest-floor dweller and grows in small clusters or individually on grasslands (Ge et al., 2010). The fructification of *M. detersa* occurs only once a year during rainy season in the month of June – August in northern Thailand.

Wild edible mushrooms are collected not only for consumption but also recognized as a good sources of digestible proteins, carbohydrates, fibres and vitamins (Barros et al., 2007b; Heleno et al., 2009; Kalac, 2009; Ouzouni et al., 2009). Similarly wild edible *Macrolepiota* species are collected during the fructification seasons and widely consumed in China, India and some parts of Thailand (Ge et al., 2010). Despite the taste, *Macrolepiota* species are also known to contain rich nutraceutical contents (Kumari and Atri, 2014). Currently, three species of *Macrolepiota* such as, *M. dolichaula*, *M. gracilenta* and *M. procera* are cultivated

in Thailand (Kwon and Thatithatgoon, 2004; Thawthong et al., 2014). Till date, there was no research carried out to cultivate *M. detersa*, which has an equal potential to domesticate.
The purpose of this research is to provide the growth parameters such as optimal culture conditions, for domestication of wild tropical edible mushroom *M. detersa.*

Methodology

Mushroom collection, Isolation and culture preparation
Macrolepiota detersa was collected from a mixed deciduous forest of Doi Mae Salong, Chiang Rai, Thailand. Morphological characters of this mushroom were recorded using the compound microscope (Carl Zeiss™SteREO Discovery.V8 Microscopes, Jena, Germany). Pure cultures were prepared using the tissues isolated from the internal part of the cap. Tissue was centrally inoculated into malt extract agar (MEA) and incubated at 30 °C for 12 days. After 12 days, the agar surface was fully covered with white mycelium. Stock cultures were prepared and kept on PDA slants at 4 °C in Mae Fah Luang University culture collection (MFLUCC) for further study.

Effects of media on mycelial growth
Nine different culture media for mycelial growth were selected such as yeast malt extract agar (YMEA), glucose peptone yeast extract agar (GYP), corn meal agar (CMA), oat meal agar (OMA), potato dextrose agar (PDA), malt extract agar (MEA), sabouraud dextrose agar (SDA), malt extract agar-sucrose (MEA-S) and malt extract yeast extract agar (MYEA) were used to determine suitable media for promoting mycelium growth. A 6.0 mm diameter mycelial plug was cut from the edge of the ten days old *M. detersa* culture grown on MEA and placed in the center of each agar plate containing the optimized media. The petri dishes containing cultures were incubated at 30 °C for 12 days. Mycelial growth was evaluated by mycelial diameter and mycelial dry weight after every two days for 12 days. All the experiments mentioned in this paper were carried out in triplicates.

Effects of different temperatures on mycelial growth
The selected medium was used for evaluation at different temperatures 16 °C, 18 °C 25 °C, and 30 °C to find out the best mycelial growth. The fungal cultures were incubated in different incubator having different temperatures for 12 days. The colony diameter and mycelial dry weight were measured and compared, to find out the optimal temperature for mycelia growth of *M. detersa.*

Effects of different pH values on mycelial growth
The selected medium and the optimum temperature were used to evaluate the optimal pH for mycelial growth. The pH were adjusted to 4.0, 5.5, 6.0, 7.0, 8.0 and 9.0 using1N NaOH or 1N HCl. The pH ranges were measured using a digital pH meter before autoclave. The best pH optimum for promoting mycelial growth was determined by measuring the colony diameter and mycelial dry weight.

Agricultural waste and cereal grains for spawn production

Nine different types of cereal grains and agricultural wastes such as red sorghum (RS), white sorghum (WS), saw dust (SD), maize corn (MC), barley grain (BG), rice hull (RH), rice bran (RB), rice straw (RS) and white bean (WB) were used to determine the best spawn production for a tropical *M. detersa*. The spawn media were prepared as described by Ashraf et al., (2013). Each cereal grains or agricultural waste was washed and soaked in distilled water overnight and boiled for 15 minutes, then left to cool down for 20 minutes. A 250 ml media bottle was filled with 20g of cereal grains and agricultural waste and autoclaved at 121°C for 15 minutes. The substrates in bottles were shaken to prevent clump formation. Mycelial plugs (6.0 mm diameter) were cut and aseptically inserted on the top of the each substrate respectively. Spawn bottles were incubated at 30°C for 14 days. The mycelium weight was evaluated after 14 days of inoculation. Uninoculated bottles with each medium were used as control.

Data collection and statistical analysis

A completely randomized design was used in this study. The data obtained for mycelial growth under different conditions were from three replicates. The mycelial growth of *M. detersa* was also determined for comparative purpose. The results were expressed as means and variance. Means were compared using Duncan's multiple range tests by using SPSS-16 program Brosius, (2008).

Results

Effect of different types of media on mycelia growth

In our study, nine different solid media were screened for the favorable growth of *M. detersa*. Although the mycelial growth was seen maximum in PDA but we have not selected it as the best media. The best medium is MEA as the best colony diameter as well as mycelial dry weight was observed on MEA (Table 1). Our results are in consistence with those of Wozniak, (2009) who identified the highest mycelial diameter of *M. procera*, on PDA.

Table 1 Effect of various solid media on mycelial growth of *Macrolepiota detersa* at 30 ºC after 12 days of inoculation.

Cultural media	Mycelial growth	
	Colony diameter (cm)	Mycelial dry weight (g)
PDA	9.00±0.00 ^{a}	0.11±0.06 ^{b}
MEA	8.43±0.40 ^{ab}	0.30±0.03 ^{a}
CMA	7.73±0.55 ^{b}	0.04±0.00 ^{cd}
OMA	2.57±0.60 ^{f}	0.02±0.00 ^{d}
SDA	5.67±0.32 ^{d}	0.06±0.02 ^{cd}
GPY	6.57±0.15 ^{c}	0.08±0.02 ^{bc}
YMEA	7.00±0.60 ^{c}	0.09±0.00 ^{b}
MEA-S	3.53±0.40 ^{e}	0.08±0.03 ^{bc}
MYEA	3.70±0.26 ^{e}	0.02±0.00 ^{d}

The results are mean and standard deviation of three replicates. Data with different letters within the same column indicated the significant difference at $P<0.05$ according to Duncan's multiple range tests.

Effect of different temperatures on mycelial growth of *Macrolepiota detersa* on MEA after 12 days of incubation

Macrolepiota detersa was tested for the suitable temperature for promoting mycelial growth on MEA medium. Temperatures: 16 ºC, 18 ºC, 25 ºC and 30 °C were used for the mycelial growth (Table 2). Mycelia grew well between 18 ºC and 30 °C and the best mycelial growth was identified at 30 °C. This finding was in agreement with the mycelial growth of *M. procera* reported by Shim et al., (2005).

Table 2 Mycelial growth of *Macrolepiota detersa* at different temperatures after 12 days of incubation. Mean and standard deviation of three replicates

	Mycelial growth	
Temperature (ºC)	Colony diameter (cm)	Mycelial dry weight (g)
30	7.47±0.35	0.31±0.00
25	7.10±0.40	0.28±0.01
18	5.57±0.15	0.25±0.01
16	4.03±0.64	0.23±0.00

Effect of different pH values on mycelial growth of *Macrolepiota detersa* on MEA medium at 30 ºC after 12 days of incubation

All pH from 4 – 9 were suitable for the mycelial growth of *M. detersa*. The optimal pH on mycelial growth was in the range of pH 7 – 8 (Table 3). Similar with our results the optimal pH for *M. procera*, *Lignosus rhinoceros*, and Thai oyster mushroom were 5–8 (Shim et al., 2005; Lai et al., 2011; Kumla et al., 2014).

Table 3 Mycelial growth of *Macrolepiota detersa* at different pH on MEA at 30 ºC after 12 days of incubation. Mean and standard deviation of three replicates

	MEA adjusted with 1M HCl and 1M NaOH	
pH	Colony diameter (cm)	Mycelial dry weight (g)
4.0	5.90±0.10	0.18±0.00
5.5	6.10±0.10	0.16±0.03
6.0	6.23±0.15	0.20±0.02
7.0	7.23±0.21	0.24±0.02
8.0	7.47±0.35	0.23±0.00
9.0	5.97±0.55	0.19±0.02

Cereal grains and agricultural waste for spawn production

Our study examined the use of different cereal grain or agricultural substrates for promoting mycelial growth for spawn production. Mycelia of *M. detersa* was able to grow on all spawn media after 14 days following inoculation. The highest mycelial weight was observed in red sorghum and barley grains after two weeks (Table 4). Similar to our result, red sorghum grains was found to be the best substrate for spawn production for Thai oyster mushroom (Kumla et al., 2014). In general, sorghum grains are used for spawn production, as it is easily available, cheap, and due to its ability to soak optimal amount of water (Narh et al., 2011).

Table 4 Effect of different types of cereal grain and agricultural waste on mycelial growth of *Macrolepiota detersa* after 14 days of inoculation. Mean and standard deviation of three replicates.

Substrates (20g)	Mycelial weight (g)
Red sorghum	2.38±0.59
Rice straw	1.28±0.41
Maize	1.70±0.14
Saw dust	1.48±0.37
White sorghum	1.34±0.48
Bean	1.78±0.34
Rice bran	1.06±0.85
Rice hull	0.14±0.07
Barley	1.99±0.38

Discussion

Unlike other saprobic mushrooms, *Macrolepiota* species are difficult to collect and make an attempt to study their ecology and diversity in detail as *Macrolepiota* species are found to grow singly at specific humidity and temperature during rainy seasons (Vellinga, 2004). Nevertheless various biological activities, including anti-microbial, antioxidant and enzyme (trypsin, monophenolase) activities of *Macrolepiota* species are reported (Vetter, 2000; Puttaraju et al., 2006; Barros et al., 2007a; Kolcuoglu et al., 2007; Dulger et al., 2008). Some edible *Macrolepiota* species are also known to contain good amounts of nutrition (Kumari and Atri, 2014). However, a few species have been studied with regard to their secondary metabolites, optimal mycelial conditions and cultivation. The results of the optimal mycelial conditions showed that *M. detersa* could be easily grown in wide ranges of media, pH, temperature and agricultural wastes in a short period of time with low contamination. The cultivation parameters of *M. detersa* should be explored in order to make it available throughout the seasons. Domestication of edible mushrooms not only supply nutritious diet for humans but also would help in conservation of valuable wild edible species of *Macrolepiota* and other mushroom species for future generation.

Conclusion

This is the first attempt to optimize the mycelial conditions of wild edible *M. detersa* in Thailand. Favorable medium, temperature and pH for the mycelia growth of *M. detersa* were observed on MEA with pH 7.0 or 8.0 at 30 ºC within a period of 12 days. Mycelia utilized all the agricultural wastes used in this experiment at different rates; however the highest mycelial weight of this fungus was seen in both red sorghum and barley grains media. Since this is the first study of optimal conditions on mycelial growth of *M. detersa*, we would like to recommend, to use MEA and PDA as culture media at 25 – 30 ºC, pH 7.0 – 8.0 and red sorghum and the barley grains as the spawn medium, for growing. Future studies will attempt to identify cultivation condition and improving the yield by varying the conditions and substrate parameters to domesticate *M. detersa* in Thailand.

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