



Isolation and Mycelial Growth of Mushrooms on Different Yam-based Culture Media

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ABSTRACT

This study reports the potential application of yams as an efficient substrate for isolating mushrooms from the fruiting body. It seems that the utilization of yams as the medium for edible mushroom cultivation, i.e. a substitute for PDA, may increase the economic value of yams. Yams (*Dioscorea* sp.) belong to the starch-rich and energy-rich tubers, together with cassava, sweet potato, and potato. There is little utilization of yams in Thailand because yams grow wild and uncultivated and thus sufficient quantities of yams are available for home consumption and in local markets. In this study, an experiment was performed to assess the suitability of yam-based culture media as a potentially high value material for edible mushroom isolation and mycelial growth as a substitute for potato dextrose agar (PDA). Yam-based culture media were prepared from three species (*D. alata* L., *D. esculenta* and *D. pentaphylla*) in varying concentrations (0, 5, 10, 15, 20, 25, and 30% (w/v)). PDA was used as the standard medium. Three edible mushrooms, i.e. *Pleurotus sajor-caju* (Fr.) Sing., *Lentinula edodes*, and *Volvariella volvacea* were investigated. Mycelial growth of the mushrooms was measured the average mycelium colony diameter on the different media. The results of mycelial growth of the mushrooms show that yam-based culture media can be used as alternative culture media for cultivation.

1. INTRODUCTION

Yams (*Dioscorea* sp.), together with cassava, sweet potato and potato, belong to the starch-rich and thus energy-rich tubers. Yams are more restricted to limited areas and well-known to villagers in the north and northeastern part of Thailand as a rice supplement. A chemical extract from some species has been found to have a potential use as an insecticide, and yams are also used as a healthy food and source of herbal medicinal ingredients [1]. Normally, it is used boiled or steamed and sometimes cooked with sugar-coconut milk. *D. alata* L., *D. esculenta*, and *D. hispida* are the most important yam species; several other species have been identified, but few have been collected. Scientists hope to collect more species as plant facilities improve. The utilization of yams for the formulation of laboratory media has been done for the cultivation of *Pleurotus*

tuber-regium (Fr.) Sing. and *P. sajor-caju* [2, 3], as well as the production of mycelial biomass, exopolysaccharides and mycelium protein [4], the isolation of tuber-rot fungi [5] and cultivated fungi [6, 7, 8], and as an efficient substrate for mushroom cultivation [9]. One of the most important general purpose agar media is Potato Dextrose Agar (PDA). The feasibility of developing alternative media to different culture media, particularly PDA, has been studied by different researchers. There is low utilization of yams in Thailand because yams grow wild and uncultivated and thus sufficient quantities of yams are available for home consumption and in local markets. This study aimed to evaluate the potential application of yams as a source of efficient substrate-modified agar media for the mycelium growth of mushrooms.

The process of culturing mushrooms has three main steps: isolating the mushroom from the fruiting body, preparing primary and secondary spawn, and culturing the mushroom from spawn to harvest fruiting bodies [10]. The aim of this study was to evaluate the effects and to find the optimal concentrations of yams that allow for mycelial growth of three mushroom species, including *Lentinula edodes*, *Pleurotus sajor-caju* (Fr.) Sing., and *Volvariella volvacea*, with the goal of promoting mycelial growth.

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2. MATERIAL AND METHODS

This investigation was carried out research laboratory of Department of Plant Science, Faculty of Agricultural Technology and Industrial Technology, Phetchabun Rajabhat University, Thailand during October to November, 2015. The details of materials and methods are described as below;

2.1 Material preparation

Three different yam species were as the substrate, i.e. *D. alata* L., *D. esculenta*, and *D. pentaphylla*, and were tested for their ability to support mycelial growth. The edible mushrooms included *L. edodes*, *P. sajor-caju* (Fr.) Sing., and *V. volvacea*, obtained from a farm located in Muang, Phetchabun, Thailand.

2.2 Yam-based culture media preparation

The different culture media, i.e. DADA (*D. alata* L. Dextrose Agar), DEDA (*D. esculenta* Dextrose Agar), and DPDA (*D. pentaphylla* Dextrose Agar) were compared with PDA (Potato Dextrose Agar) for mycelial growth. The yam-based culture media were used in different concentrations at 0-30% (w/v) of each yam species. The skin was peeled from 500 g of yams and cut into small pieces, then 300 g of each yam and 200 g of potato was carefully weighed. They were then boiled in 500 ml of water for 15 min. The yam extract was prepared by filtering through muslin cloth and then used for medium preparation. The extract was made up to 1,000 ml using distilled water. The composition of the medium included 20 g of dextrose and 15 g of agar for solid media. An Erlenmeyer flask containing 150 ml of medium was cotton plugged and autoclaved at 121 °C for 15 min. After cooling to about 45°C, it was then dispensed into Petri dishes in duplicate.

2.3 Mushroom culture

Fresh cultures of *L. edodes*, *P. sajor-caju* (Fr.) Sing., and *V. volvacea* were cut with sterilized blades, then a small piece of the mushroom tissue was removed and placed on PDA medium and incubated at 25°C for 10 days. Several sub-cultures were made until pure cultures were obtained. Mycelium discs (5 mm in diameter) from seven-day-old mushroom cultures were inoculated in each Petri dish (placed in the center of the medium) and incubated at 25°C for seven days in darkness. The diameter of the mycelium expansion was measured for seven days and colony morphology was recorded.

2.4 Measurement of mycelial growth

The vegetative growth of the mycelium of the mushroom on the different media was assessed by measuring the diameter of mycelium in the Petri dish prior to inoculation.

2.5 Mother spawn preparation

Sweet sorghum seeds as the spawning material to support extensive mycelial growth were washed in clean water three times to remove dust and other particles. The grains were then soaked in water for 24 h for maximum absorption of water. Soaked grains were again washed in water drained and put into spawn bottles. About 2/3 of each spawn bottle were filled with grains after which they were autoclaved at 121°C for 15 min. The grains in the bottles were then inoculated with five mycelium discs (5 mm in diameter) per bottle under aseptic conditions. They were incubated at 30°C for 14 days. Inoculated bottles were gently shaken for 5 and 10 days.

3. RESULTS AND DISCUSSION

The present study points to the importance of an underutilized tuber crop such as *D. alata* L., *D. esculenta*, and *D. pentaphylla* (Fig. 1) on mycelial growth for commercial applications. There were three culture media which cultured mushrooms successfully in the laboratory environment. All the tested materials showed positive results for mycelial growth, which are provided in Table 1. The mycelial extension of *L. edodes*, *P. sajor-caju* (Fr.) Sing., and *V. volvacea* was compared on different culture media. The colony characteristics of all three mushroom species grown on yam-based culture media were assessed for 7 days.

The highest mycelial growth of *V. volvacea* was 9.00±0.00 cm on DPDA-30; for *P. sajor-caju* (Fr.) Sing. This was 5.47±0.42 cm and for *L. edodes* this was 3.73±0.06 cm on DADA-20 and DADA-25, respectively (p<0.05) (Table 1). To determine the optimal concentration of yams for mycelial growth, the concentration of 5-30% (w/v) DADA and DPDA gave the greatest mycelium colony diameter (2.50±0.00 to 9.00±0.00 cm.). The highest mycelium colony diameter of *L. edodes* (3.73±0.06 cm.) cultured on DADA was achieved at 20-25% (w/v). However, the highest concentration of DEDA medium was unable to increase the mycelium colony diameter. It is thought that the type of yam and or a high concentration of yam-based culture medium may inhibit mycelial growth.



Fig. 1: Showing different tubers and leaves of three *Dioscorea* sp. A) *D. alata* L. B) *D. esculenta* and C) *D. pentaphylla*

Table 1: Effect of different culture media on the mycelial growth of mushrooms cultured for 7 days.

Culture media	Concentration (% w/v)	Mycelium colony diameter (cm)		
		<i>L. edodes</i>	<i>P. sajor-caju</i> (Fr.) Sing.	<i>V. volvacea</i>
DADA	0	1.57±0.15 ^{fg}	2.43±0.40 ^{de}	6.14±0.25 ^d
	5	2.60±0.17 ^{de}	3.30±0.26 ^{cd}	4.93±0.21 ^e
	10	3.10±0.30 ^{bc}	4.43±0.38 ^b	6.23±0.25 ^d
	15	3.27±0.06 ^b	4.10±0.56 ^{bc}	3.57±0.32 ^{fg}
	20	3.37±0.58 ^a	4.67±0.35 ^{ab}	4.70±1.04 ^e
	25	3.73±0.06 ^a	4.40±0.89 ^b	4.10±0.53 ^{ef}
DEDA	0	1.67±0.06 ^{fg}	2.03±0.25 ^{ef}	0.50±0.00 ^j
	5	1.20±0.17 ^h	0.50±0.00 ^h	4.20±0.53 ^{ef}
	10	1.23±0.06 ^{gh}	2.73±0.40 ^{de}	3.57±0.15 ^{fg}
	15	0.83±0.06 ^{jk}	3.13±0.64 ^d	2.70±0.17 ^h
	20	0.63±0.06 ^k	2.67±0.42 ^{de}	2.43±0.32 ^{hi}
	25	0.70±0.17 ^k	1.97±0.15 ^{ef}	2.97±0.06 ^{gh}
DPDA	0	1.33±0.25 ^{ij}	0.50±0.00 ^{gh}	1.83±0.15 ^j
	5	2.50±0.00 ^e	4.37±0.49 ^b	8.23±0.21 ^{ab}
	10	2.77±0.06 ^{cd}	4.33±0.21 ^b	7.63±0.21 ^{bc}
	15	2.93±0.23 ^{bcd}	3.30±0.17 ^{cd}	7.37±0.40 ^c
	20	2.63±0.06 ^{de}	4.27±0.83 ^b	7.20±0.69 ^c
	25	2.87±0.29 ^{de}	5.47±0.42 ^a	7.63±0.32 ^{bc}
PDA	0	2.93±0.12 ^{bcd}	4.17±0.76 ^{bc}	9.00±0.00 ^a
	20	3.27±0.56 ^b	3.10±0.10 ^d	7.20±0.26 ^c

DADA: *D. alata* L. Dextrose Agar; DEDA: *D. esculenta* Dextrose Agar;

DPDA: *D. pentaphylla* Dextrose Agar; PDA: Potato Dextrose Agar.

Means within the same column followed by the same letters are not significantly different at $p < 0.05$. Each value is expressed as mean±standard deviation (SD), $n=3$.

The observed mycelium colony diameter of mushrooms on different media was significantly different at $p < 0.05$. The results of the present research show that the mycelia of *L. edodes* and *P. sajor-caju* (Fr.) Sing. formed on DADA and DPDA faster than on DEDA medium. However, the difference in the average mycelial growth between PDA and DPDA was not significant ($p > 0.05$). The mycelia that grew on DADA, DEDA and DPDA were thinner than on PDA (Fig. 2). It seems that the colony characteristics of the mycelium depend on the medium composition, the efficacy of biocompounds, the type of yam species and the concentrations of the substrate [4, 11, 12].

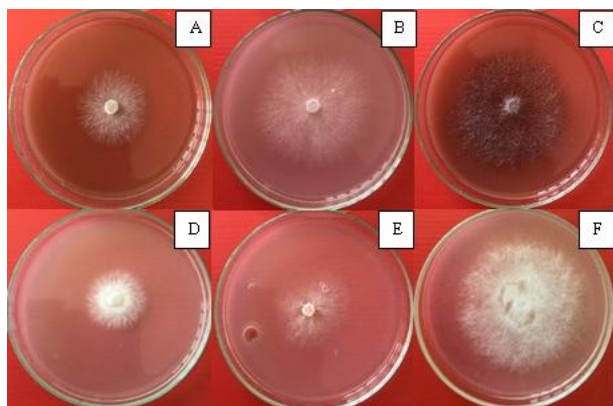


Fig 2: Morphology of mycelia growth of *V. volvacea* (A), *P. sajor-caju* (Fr.) Sing. (B), and *L. edodes* (C) cultured on yam-based culture media and *V. volvacea* (D), *P. sajor-caju* (Fr.) Sing. (E), and *L. edodes* (F) cultured on PDA for 7 days (bar = 0.5 cm).

In this study, it was evident that some media supported the growth of some strains of mushroom better, which means that each needs to be studied individually. Fai et al. [13] applied a

commercially available culture media of yam bean as a tuber substrate to produce chitin and chitosan by *Mucor circinelloides*. This is in agreement with the findings of Vikraman et al. [8] who reported that tuber crops, especially *Dioscorea*, are bestowed with an immense starch content, which can be used as a food source for fungal culture. Hoa and Wang [14] reported the use of alternative culture media for growing oyster mushrooms (*P. ostreatus* and *P. cystidiosus*). Mother spawn production as a pure culture of mycelium growing on a solid substrate such as cereal grain has a fundamental role in agricultural productivity. In this study, *V. volvacea* and *P. sajor-caju* (Fr.) Sing. were fully colonized by vigorously growing mycelia on sweet sorghum as the spawn after 14 days of incubation (Fig. 3); *L. edodes* was the exception. Stanley and Awi-Waadu [15] reported a trend for the duration of incubation on different types of grain type on mycelial fresh extension.



Fig 3: Spawn grains of *V. volvacea* (A), *P. sajor-caju* (Fr.) Sing. (B), and *L. edodes* (C) cultured on sweet sorghum grains.

4. CONCLUSIONS

This study reports the potential application of yams as an efficient substrate for isolating mushrooms from the fruiting body. Yam-based culture media of DPDA was found suitable for mycelium growth for *P. sajor-caju* (Fr.) Sing and *V. volvacea*. It seems that the utilization of yams as the medium for edible mushroom cultivation, i.e. a substitute for PDA, may increase the economic value of yams. Further research is still needed to assess the application of temperature and nutritional conditions on the mycelial growth of mushrooms and to determine the effects of substrates for spawn preparation on the mycelial growth of three mushrooms.

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Conflict of Interests: There are no conflicts of interest.

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