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THE FIRST REPORT ON THE SUCCESSFUL RESCUE AND DOMESTICATION OF PHILIPPINE WILD MUSHROOM *Oudemansiella canarii* (BIL 9137)

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ABSTRACT

This paper reported for the first time the rescue and domestication of Philippine wild strain of *Oudemansiella canarii*. The fruiting body was collected from the forested area of Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. It has flat, smooth and sticky (when moist) surface, grayish white brittle pileus with white spore print and ring-like hyaline basidiospores. Its mycelia were successfully rescued and luxuriantly grew in malt extract agar (commercially available media) and potato broth sucrose agar (indigenous media) with the highest mycelial diameters of 64.83 mm and 84.17 mm with very thick mycelial density after 6 days of incubation, respectively. Both sorghum seeds and cracked corn as grain spawn materials registered the shortest period of incubation of 11.33 days with very thick mycelial density. Substrate formulation of 8 parts rice straw + 2 parts sawdust significantly produced the highest yield of 89.67 g and recorded the highest biological efficiency of 18.94%. This study also documented the different stages of fruiting body formation including primordial initiation, button stage, stipe elongation, pileus expansion, and maturation stage.

Keywords: Fruiting body, *Oudemansiella canarii* (BIL 9137), rescue and domestication

INTRODUCTION

Mushroom is a basidiomycetous fungus which naturally occurring on lignin-cellulosic substrates in the temperate and tropical part of the world. It is one of the most diverse organisms during rainy season where moisture triggers their proliferation. The Philippines is rich in mycological resources that need to be rescued from the wild. One of wild species of mushroom is the *Oudemansiella canarii*, an edible basidiomycetes belong to the family Physalacriaceae. This mushroom is commonly found growing on trunks and twigs of dead trees, fallen logs, and pile of wood chips. Its pileus is bell-shaped with varying from dark brown at young and becoming flat, grayish to yellowish white when matures. The surface is smooth and sometimes wrinkled on disc (center) and inner limb and slimy to sticky when moist. Previously, we have identified and species listed *O. canarii* as one of the naturally occurring mushrooms in the multistorey agroforestry systems in Mt. Makiling Forest Reserve, Los Baños, Laguna, Philippines **[1]** but the mycelia were not successfully rescued. Recently, a wild fruiting body of *O. canarii* was found growing on the fallen log in the forest area of Lingap Kalikasan, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines and its mycelia were grown in mycological media.

We report here the successful rescue, domestication, and cultivation of the Philippine wild mushroom *O. canarii*. The growth performance of its secondary mycelia was evaluated on different indigenous culture media and commercially available mycological media. The different granular materials were evaluated as spawning materials and the fruiting body production was determined in different formulations of rice straw and sawdust in artificial cultivation. Therefore, this study demonstrated the production technology of Philippine wild *O. canarii.* However, we are currently conducting optimization studies in order to improve the yield and biological efficiency of this valuable edible mushroom.

MATERIALS AND METHODS

Source of Strain

Wild strain of fruiting body of *O. canarii* was collected from the forest area of Lingap Kalikasan of Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. The wild fruiting body, spore print and microspic spores of *O. canarii* are shown in Figure 1.

Mushroom Tissue Culture

The collected fruiting body of *O. canarii* was brought in the laboratory for tissue culture. Sterile tissues from the internal part of the fruiting body were cut using sterile surgical blade and inoculated onto commercial medium Potato Dextrose Agar (PDA) plates. Inoculated plates were incubated in biological incubator (32°C) for 7 days to allow mycelial ramification. Culture plates were coded as BIL 9137 and were used as inoculants for the evaluation mycelial growth and fruiting body production.

Evaluation of Secondary Mycelial Growth

The secondary mycelial growth performance of *O. canarii* on different culture media was evaluated. Three commercially available culture media namely; malt extract agar, potato dextrose agar, and Sabouraud dextrose agar and four indigenous culture media including coconut water agar (from mature coconut water), rice bran broth agar (broth from 5% D1 rice bran), corn grit broth agar (broth from 5% local yellow corn grit), and potato sucrose agar (broth from 25% potato) were used in the evaluation. The prepared culture media were sterilized at 15 psi, 121 °C for 20 min and allow to cool before pour plating in sterile petri plates. Culture media were inoculated centrally with 10 mm-diameter mycelial discs from seven-day old pure plate culture of *O. canarii*. The inoculated plates were sealed with parafilm and incubated at 32 °C under alternating light and dark conditions. The diameter of mycelial growth was measured until fully ramification. Each set-up was replicated three times.

Evaluation of Spawning Material

The growth performance of *O. canarii* secondary mycelia in three granulated materials such as cracked corn, rice seeds, and sorghum was also evaluated. Grains were boiled until swelling and tender. These were drained and air dried until 65% moisture content was attained and 40 g of each were dispensed into clean glass bottle. Each treatment was replicated three times. The bottled substrate were plugged with cotton, wrapped with clean paper and sterilized at 15 psi or 121 °C for 45 min. After sterilization and cooling, the sterile bottled grain were inoculated with 10 mm mycelial disc from the seven-day old pure culture and incubated at room temperature (32 °C) to allow mycelial growth and ramification. Number of days of full mycelial colonization and the mycelia density were recorded. The substrate with the shortest period of incubation until fully ramification and most luxuriant mycelia was the best spawning material.

Evaluation of Fruiting Body Production

Rice straw and sawdust were the substrates used in the evaluation of fruiting body production of *O. canarii*. The rice straw was soaked for 3 days in water tank and then washed out to remove undesirable odor. This was hauled out, piled, and covered with plastic sheets to decompose for three days. Then, the rice straw was chopped about 30-50 mm lenght and mixed with parts of sawdust with 65% moisture content. Five hundred grams of formulated substrates were bagged and compacted in a 6 × 12-inch polypropylene bag. Each treatment was replicated 5 times. The prepared bags were plugged with cotton, fastened with polyvinylchloride necks and sterilized at 121 °C, 15 psi for 1 h. After cooling, 40 g of best spawn material (from the previous sub-study) was inoculated into each sterile bag. The inoculated bags were incubated at 32 °C to allow growth and full ramification of mycelia. The number of days of total mycelia ramification on different rice-straw based substrates was recorded. Fruiting bags were opened to allow primordial development and fruiting body maturation. Matured fruiting bodies were harvested and weighed and the percentage biological efficiency of *O. canarii* was calculated.

Statistical Analysis

All treatments were laid out in a Complete Randomized Design (CRD). Data were analyzed using analysis of variance (ANOVA) in one way classification analysis. The treatment means were compared using Duncan Multiple Range Test (DMRT) at 5% level of significance. The SAS statistical program was used for analysis.

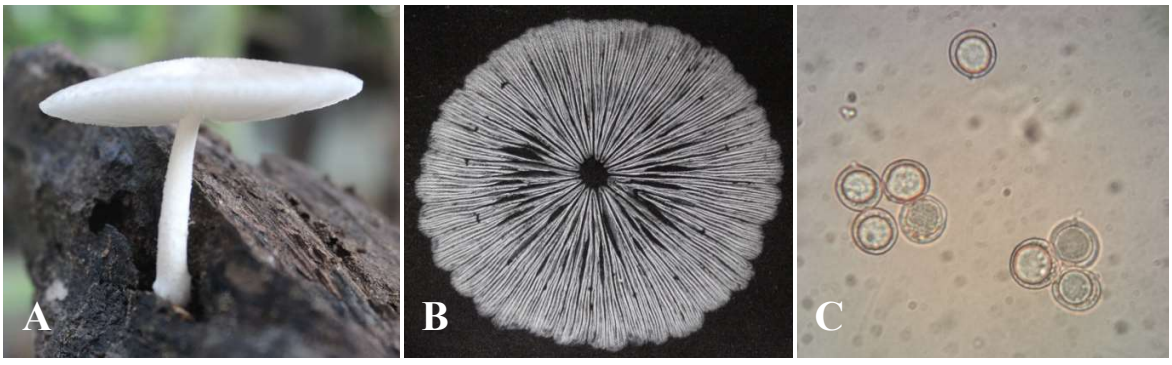

Figure 1: Wild fruiting body (A), spore print (B) and basidiospores (C) of *Oudemansiella canarii*.

RESULTS AND DISCUSSION

Mycelial Growth on Mycological Culture Media

The collected fruiting body of *Oudemansiella canarii* (Figure 1A), from the forest area of Lingap Kalikasan, Central Luzon State University, has flat and smooth surface (sticky when moist), grayish white brittle pileus. It has white spore print and ring-like hyaline basidiospores (Figure 1). This was successfully rescued and grown in potato dextrose agar and the culture was labeled as BIL 9137. The culture medium preference was evaluated in order to produce healthy cell lines in pure culture, which will serve as source of mycelia for biomass production leading towards the medicinal and pharmacological evaluation. In this study, the mycelial growth performance of *O. canarii* on the three commercially available and four indigenous culture media was evaluated based on mycelial growth diameter and density. Table 1 presents the results of mycelial growth evaluation. Apparently, mycelia of *O. canarii* respond differently on the evaluated mycological media. Malt extract agar, among the commercially available culture media, significantly produced the highest mean mycelial diameter of 64.83 mm after 6 days of incubation, followed by Sabouraud dextrose agar with 55.50 mm growth diameter. Contrastingly, potato dextrose agar had the lowest mean mycelial growth of 52.17 mm. The slower mycelial growth in Sabouraud dextrose agar indicates the preference of *O. canarii* mycelia to low peptone. The three media showed very thick mycelial growth (Figure 2). On the other hand, among indigenous culture media, the fastest rate of mycelia growth was recorded in potato broth sucrose agar with a mean 84.17 mm after 6 days of incubation and produced a very thick mycelial density. Surprisingly, this indigenous medium is more preferred than the commercially available potato dextrose agar for the efficient mycelial growth of *O. canarii*, suggesting that dextrose is too nutritious for *O. canarii* that probably limits its mycelia to extend. The lowest mean mycelial diameter was significantly recorded in rice bran broth agar with 60.00 mm mean mycelial diameter with only thick mycelial density. In our previous evaluation of other wild mushrooms, *Lentinus strigosus* mycelia favored Sabouraud dextrose agar and coconut water agar **[2]** while the luxuriant mycelial growth of *Polyporus grammocephalus* was obtained in malt extract agar and corn grit broth agar **[3]**. These findings clearly indicate that each species of mushroom has unique culture medium preference.

Table 1: Mean diameter of mycelial growth of *Oudemansiella canarii* on different culture media under different length of incubation and its mycelia density

Culture Media	Diameter of Mycelial Growth (mm)			Mycelial Density
	Day 2	Day 4	Day 6	
Malt Extract Agar	14.50 ± 0.50^{ab}	36.67 ± 2.31^{a}	64.83 ± 2.08^{a}	++++
Potato Dextrose Agar	14.67 ± 0.58^{a}	32.83 ± 1.04^{b}	52.17 ± 1.26^{c}	++++
Sabouraud Dextrose Agar	13.50 ± 0.50^{b}	32.33 ± 1.89^{b}	55.50 ± 0.50^{b}	++++
Coconut Water Agar	15.17 ± 1.04^{a}	47.33 ± 2.93^{a}	77.33 ± 3.75^{b}	++++
Corn Grit Broth Agar	11.33 ± 0.29^{c}	37.33 ± 4.73^{b}	68.33 ± 6.75^{c}	+++
Potato Broth Sucrose Agar	14.00 ± 0.87^{b}	49.50 ± 7.94^{a}	84.17 ± 5.35^{a}	++++
Rice Bran Broth Agar	12.00 ± 1.00^{bc}	37.50 ± 0.87^{b}	60.00 ± 0.00^{d}	+++

Values are mean ± SD of three replicates. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance. In a column mycelial density: very thin (+), thin (++), thick (+++), very thick (++++).

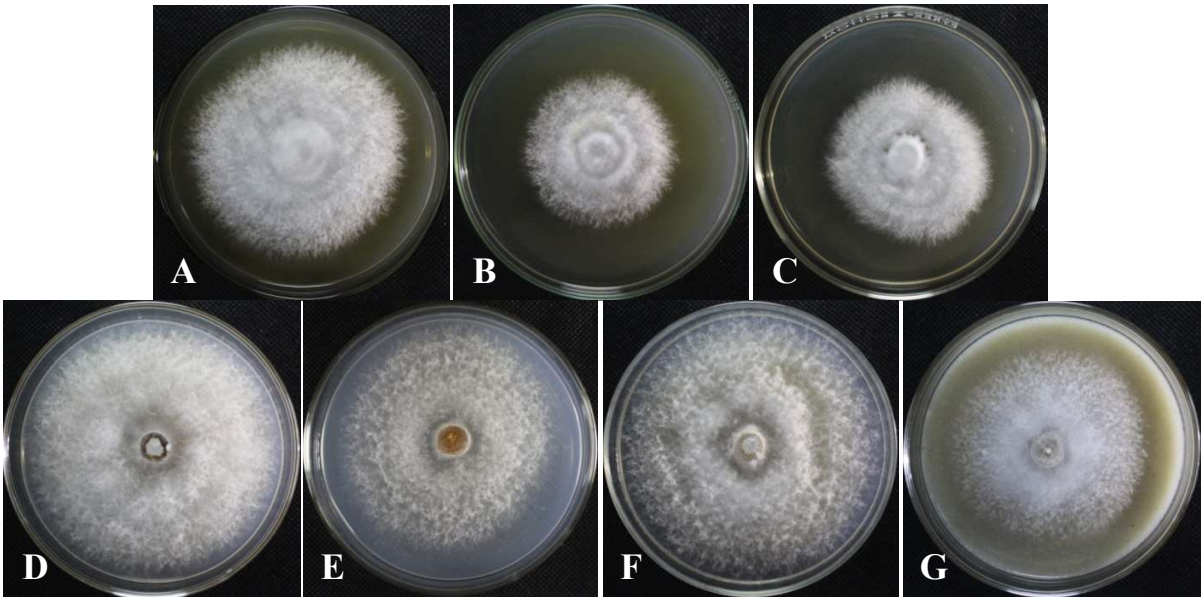

Figure 2: Mycelial growth performance of *Oudemansiella canarii* on the different culture media: (A) malt extract agar (B) potato dextrose agar; (C) Sabouraud dextrose agar; (D) coconut water agar; (E) corn grit broth agar; (F) potato broth sucrose agar and (G) rice bran broth agar after 6 days of incubation.

Growth Performance on Grain Spawn

The grain spawn is the inoculant used in fruiting body production. The mycelia block of mushroom is inoculated into the different granulated spawning materials such as cracked corn, rice seeds, and sorghum seeds. The number of days of full mycelial ramification from the time of inoculation was determined. The incubation period and mycelial density of *O. canarii* in the three spawning materials are shown in Table 2. Sorghum seeds and cracked corn registered

the shortest period of incubation of 11.33 days with very thick mycelial density (Figure 3) while rice seeds recorded the most extensive incubation period of 15.00 days with very thin mycelial growth. Very thin growth of mycelia was observed in rice seeds due to the thick husk that covers the nutritious part but eventually became very thick once the mycelia penetrated to the inner portion. In this study, rice seed was used to spawn the fruiting bags for fruiting body production because of its availability and granulated characteristic that makes it easy to inoculate.

Table 2: Incubation period and mycelia density of *Oudemansiella canarii* in the different spawning materials

Grain Spawn	Incubation Period (day)	Mycelial Density
Cracked corn	11.33 ± 0.58[a]	++++
Rice Seeds	15.00 ± 0.00[b]	+
Sorghum Seeds	11.33 ± 0.58[a]	++++

Values are mean ± SD of three replicates. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance. In a column mycelial density: very thin (+), thin (++), thick (+++), very thick (++++).

Figure 3: Mycelial ramification of *Oudemansiella canarii* in the different spawning materials: (A) cracked corn, (B) rice seeds, and (C) sorghum seeds 11 days after inoculation.

Fruiting Body Performance of *O. canarii* on Rice Straw-Sawdust Based Formulation

To determine the cultivation potential of *O. canarii*, its fruiting body performance on rice straw-sawdust based formulation was also investigated. The mycelial response in the different formulations of substrate was primarily determined based on the incubation period. As shown in Table 3, the shortest period of incubation was observed in 6 parts rice straw + 4 parts sawdust with a mean of 24 days. The most extensive period of incubation was registered in pure sawdust

with 33 days. The mycelial growth of *O. canarii* in the different substrate formulations is shown in Figure 4. In terms of yield and biological efficiency of *O. canarii* (Table 3), 8 parts rice straw + 2 parts sawdust significantly produced the highest yield of 89.67 g and recorded the highest biological efficiency of 18.94%. This was followed by 6 parts rice straw + 4 parts sawdust with 74.37 g yield and 15.88% biological efficiency. The lowest yield and biological efficiency was registered in pure sawdust. The fruiting bodies of *O. canarii* grown on the different substrate formulations are shown in Figure 5. The maximum yield and biological efficiency obtained in this study are lower when compared with the yield and biological efficiency of cultivated mushrooms such as *Ganoderma lucidum*, *Pleurotus florida, Pleurotus ostreatus, Lentinula edodes, Volvariella volvacea*, *Lentinus tigrinus*, and *Auricularia* species. Therefore, optimization studies and enriched cultivation must be conducted in our intention to improve the production performance of this newly recorded wild edible mushroom.

Table 3: Incubation period, total yield of fruiting bodies per bag and percentage biological efficiency of *Oudemansiella canarii* grown in the different substrate formulations of rice straw and sawdust

Formulated Substrates (Rice straw : Sawdust)	Incubation Period (day)	Total Yield / bag (g)	Biological Efficiency (%)
10 : 0	25.67 ± 1.53^{cd}	53.70 ± 6.22^{c}	12.08
8 : 2	27.00 ± 0.00^{bc}	89.67 ± 6.82^{a}	18.94
6 : 4	24.00 ± 0.00^{d}	74.37 ± 5.56^{b}	15.88
4 : 6	26.00 ± 1.73^{bc}	57.57 ± 9.55^{c}	13.56
2 : 8	26.33 ± 0.58^{bc}	40.63 ± 4.44^{d}	7.14
0 : 10	33.00 ± 1.00^{a}	32.80 ± 5.28^{e}	5.70

Values are mean ± SD of three replicates. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance.

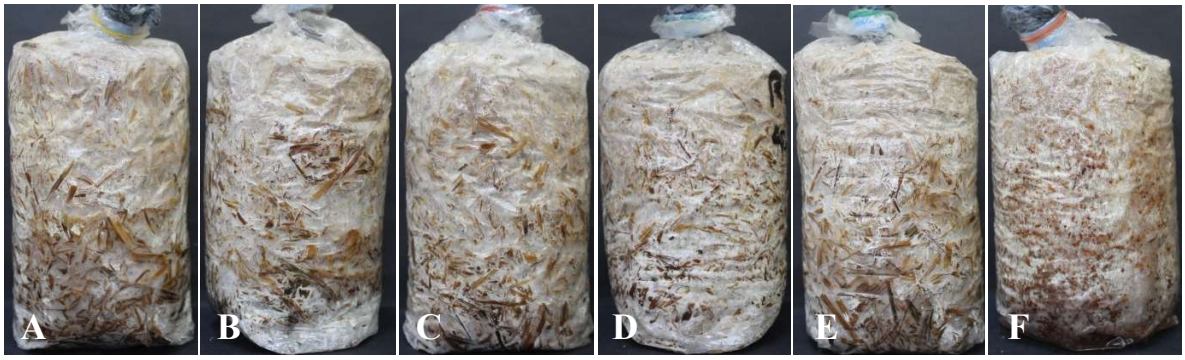

Figure 4: Mycelial growth of *Oudemansiella canarii* in the different substrate formulations (rice straw : sawdust ratio): (A) 10:0, (B) 8:2, (C) 6:4, (D) 4:6, (E) 2:8, (F) 0:10 after 30 days of incubation period

Fruiting Body Development

The developmental stages of fruiting body formation of *O. canarii* (Figure 6) were also documented in this study. The different stages of fruiting body formation include (1) primordial initiation, (2) button stage, (3) stipe elongation stage, (4) pileus expansion stage, and (5) maturation stage. After providing the favorable conditions for fruiting body development, mycelial thickening was observed in the fruiting bags. The primordia initiated from the tip of thickened mycelial coat and continued to develop to the button stage of fruiting body with dark brown shinny surface pileus. The stipe elongated and the in-rolled downward margin of pileus expanded to increase in size to become mature fruiting bodies. The mature fruiting body liberated the haploid basidiospores for new generation.

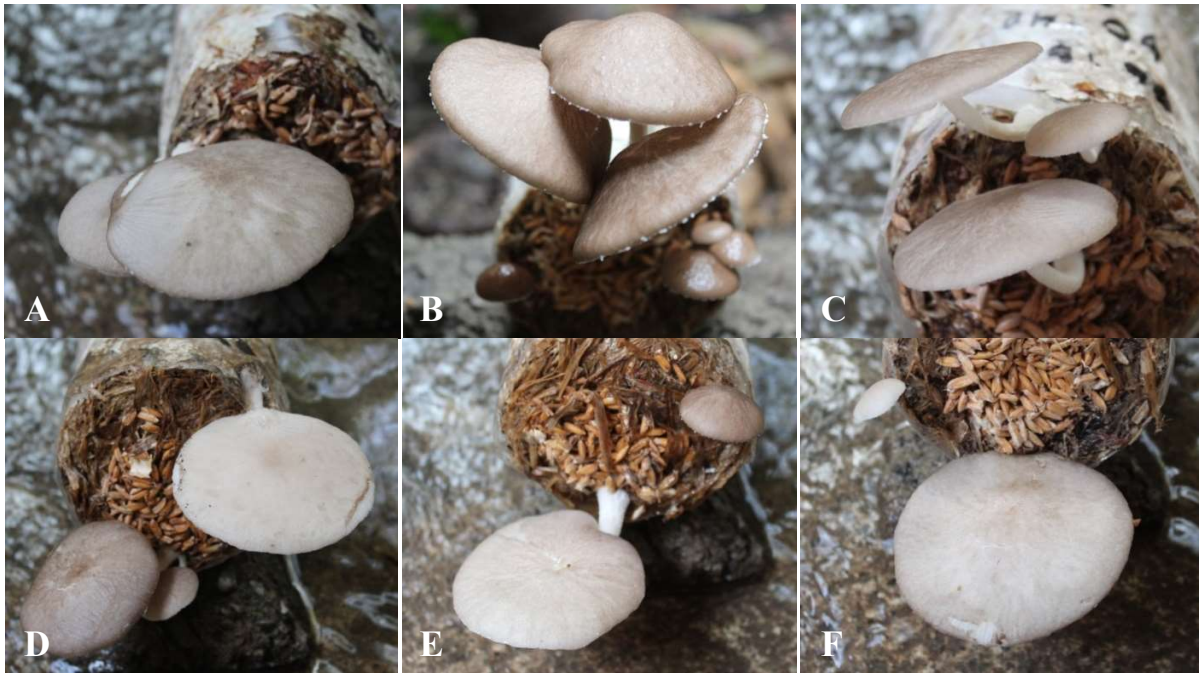

Figure 5. Fruiting bodies of *Oudemansiella canarii* grown on the different substrate formulations (rice straw: sawdust ratio): (A) 10:0, (B) 8:2, (C) 6:4, (D) 4:6, (E) 2:8, and (F) 0:10.

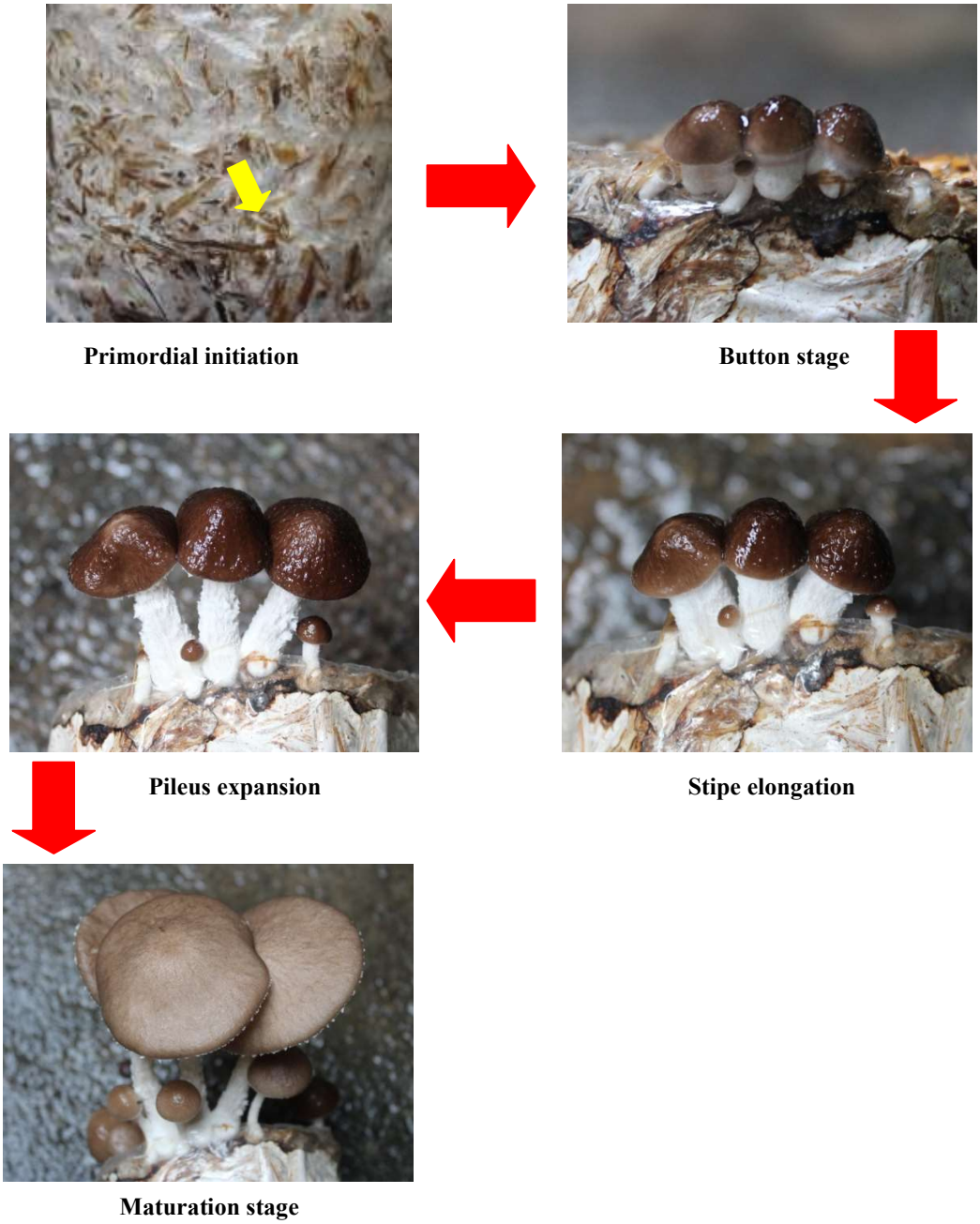

Figure 6: Development of *Oudemansiella canarii* from primordia to full grown fruiting bodies

CONCLUSION

This paper demonstrated the successful rescue and domestication of the Philippine wild strain of *O. canarii*. Malt extract agar and potato broth sucrose agar favored the luxuriant mycelial growth while sorghum seeds and cracked served as the best grain spawn materials. The maximum yield and biological efficiency of *O. canarii* was produced in 8 parts rice straw + 2 parts

sawdust. Therefore, *O. canarii* is a new record of Philippine wild mushroom that can be cultivated in laboratory conditions suggesting its promising potential for mass or commercial production. We are currently optimizing the culture conditions with reference to the nutritional and physical requirements for mycelial growth and the cultivation of fruiting body using enriched substrate in the intention to improve its biological efficiency.

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