Optimization of mycelial growth and cultivation of fruiting body of Philippine wild strain of *Ganoderma lucidum*

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Abstract

    Ganoderma lucidum is a white rot basidiomycete that grows on logs. Taking it as source of novel mycochemicals, the present study optimized growth conditions and fruiting body production of *G. lucidum* on different culture media, physical parameters like pH, aeration, illumination and temperature, spawn materials and rice straw–sawdust based substrate formulations. After 5 days of incubation, coconut water gelatin of pH 6.0 and in sealed and lighted conditions at room temperature (32°C) yielded the most efficient mycelial growth. Among the grains evaluated, corn grit produced a luxuriant mycelial growth in the shortest period of 5 days. Substrates having 70% rice straw and 30% sawdust recorded the shortest incubation period of 17.33 days for fructification. Basidiospores were germinated efficiently in coconut water gelatin after 72 hours of incubation. The basidiospores have a typical type of germination wherein the sporoderm produced a single germ tube, elongated, septated into a hypa, and branched to become monokaryotic primary mycelia. Mycelial coat hardening, primordial initiation, antler-like formation and basidiocarp maturation and spore liberation were observed as the sequence of fruit body development.

Keywords – basidiospore germination – cultivation phases – *Ganoderma lucidum* – mycelia growth – wild nutraceutical mushrooms

Introduction

    The Philippines is very rich with natural resources and remains one of the biodiversity hotspots in the planet (Myers et al. 2000). Particularly, Mt. Palali in Quezon, Nueva Vizcaya persists to date a primary forest, although anthropogenic activities could threaten the biodiversity of the place. The presence now of diverse vegetation in Mt. Palali serves as a haven of mycoflora. Though mushrooms in other countries function as one of the primary forest products and considered as prime commodity, these remained to be underutilized in the Philippines (Reyes et al. 2010). With the prevailing conditions in the country during rainy season, mushrooms became seasonally abundant and are customarily collected from the wild by the villagers. But due to the change of habitat as the end result of deforestation, climatic change and massive collection, occurrence of wild mushrooms started to diminish.

On this scale, it is essential to constantly search for mycological wild genetic resources as hearts of cell lines for more sustained research efforts towards their wise utilization and conservation. As yet, a number of mushrooms with nutraceutical potentials were conserved from the forest and suitable cultivation techniques were practically generated (Dulay et al. 2012a, 2012b, 2012c). One of the wild mushrooms having a great potential for cultivation is *Ganoderma lucidum* which has a plethora of highly important biologically active metabolites, viz.: β–glucan and ganoderic acid, synthesized in lowering the risks of arthritis, bronchitis, diabetes, hepatitis, anorexia, asthma, insomnia, leucopenia, ulcer and even the malignant cancer (Benzie et al. 2004; Stamets 2000). Treasured as divine, *G. lucidum* is a representation of good fortune, longevity and thus dubbed as the "mushroom of immortality" (Chen & Miles 1996).

Kabuteng kahoy as it is locally recognized, *Ganoderma* is a large, dark reddish–brown basidiomycete with glossy exterior belonging to the family *Polyporaceae* (or *Ganodermaceae*), order *Aphyllophorales*, class *Hymenomycetes* (Chang & Miles 2004). As this lignicolous fungus is tough to be edible, it is intended exclusively as medicine (Smith et al. 2002). Thus far, herbal medicines and dietary supplements in the forms of capsule, liquid extract and powder are manufactured and released in the market industry.

Recent studies focused on the advancement of state–of–the–art cultivation technology for *G. lucidum* to laboratory scale. Nevertheless, current state of knowledge on the physiological events in relation with its growth morphology as a prelude to developing economical large scale processes is lacking (Mitchell et al. 2003, Wagner et al. 2004). In as much as Smith et al. (2002) stressed that every fragment of the mushroom ranging from its spore to its fruiting body is extracted for health reasons, it is imperative to search for optimal nutritional and physical parameters for basidiospore germination, mycelial growth and basidiomata formation of *G. lucidum*. Another reason as emphasized by Stamets (2000) is that despite resemblance of species, growth performance always varies within the strain. Several studies were dedicated on this premise. Wang et al. (2012) examined morphological and molecular aspects of existing *G. lucidum* in China while Lee et al (2008) optimized mycelial growth of Korean strains of *G. lucidum*. Similarly, Erkel (2009) and Gurung et al. (2012) studied different substrate constitution on the production of *G. lucidum* in Turkey and Nepal, respectively.

Moreover, technological advancements allowed the controlled production of *G. lucidum* on lignocellulosic substrates such as agro–industrial residues for nutrition which supports its growth, development and fructification (Chang & Miles 2004). *Ganoderma* species have specific requirements for growth varying from environmental conditions such as pH and temperature to medium composition containing carbon and nitrogen sources. Correct selection of mushroom cultivation parameters is crucial for the development of industrial–scale cultures of *G. lucidum* (Elisashvili 2012). Hence, it is a prerequisite to design an optimal production medium and optimal process operating conditions. Thus, the desire to produce quality mushrooms while embracing traditional methods of optimizing the culture medium on selected major crops of Nueva Vizcaya, viz.: *Cocos nucifera*, *Ipomoea batatas*, *Oryza sativa, Sorghum bicolor* and *Zea mays*, necessitated for alternative substrate for *Ganoderma* cultivation is being envisioned.

With the foregoing review, elucidating the biophysiological profile of Philippine strain of *G. lucidum* from Mt. Palali with special emphasis on its morphogenesis will serve as a benchmark towards its successful production technology for utilization in the country and in Nueva Vizcaya predominantly.

Materials and methods

Source of strain

Wild strain of *G. lucidum* basidiocarp was collected from Mt. Palali, Quezon, Nueva Vizcaya and brought to laboratory to rescue the mycelia. Internal tissues from the premature basidiocarp were obtained and inoculated onto potato dextrose agar (PDA) plates with 0.1 mg/ml streptomycin

sulphate. Culture plates were incubated at room temperature to allow mycelial ramification for 7 days.

Evaluation of the mycelial growth

The growth performance of the secondary mycelia was evaluated in the different indigenous culture media, namely: coconut water gelatin, CWG, (from mature *Cocus nucifera*); corn grit decoction sucrose gelatin, CGDSG, (50 g of *Zea mays*/L of water); rice bran decoction sucrose gelatin, RBDSG, (50 g of *Oryza sativa* bran/L of water); sorghum seed decoction sucrose gelatin, SSDSG, (50 g of *S. bicolor* seeds/L of water) and sweet potato gelatin, SPG, (250 g of *Ipomea batatas*/L of water). The prepared decoctions were restored with water to make up 1 L and boiled again. Sucrose (10 g) and shredded gelatin (20 g) were added into each decoction. In coconut water gelatin, 20 g shredded gelatin was added. The culture media were sterilized at 15 psi, 121°C for 15 min and aseptically pour plated in sterile Petri plates. Mycelial discs (10 mm diameter) from a 7-day-old pure culture of the secondary mycelia were inoculated centrally onto the plated culture media. Plates sealed with parafilm and kept at room temperature under constant day and night regime. The influence of the different indigenous culture media on the mycelial growth rate of *G. lucidum* was measured every 24 hours using a digital Vernier caliper for 5 days. Mycelial biomass was also gathered following the procedure of Sánchez & Viniegra–González (1996). The media with mycelia were melted in an oven for 1 minute. The mycelia were gently removed using tweezers and rinsed in two recipients of distilled water. The mycelia were oven–dried at 60°C for 5 min and weighed using analytical balance.

Coconut water gelatin was adjusted to different pH levels (6.0, 6.5, 7.0, 7.5 and 8.0) using 0.1M NaOH or 0.1M HCl prior to sterilization. Plated media were individually inoculated with 10 mm diameter fungal disc from a 7-day-old pure culture, incubated and evaluated. From the most appropriate pH level, inoculated plates were sealed using parafilm and the other plates remain unsealed to evaluate the influence of aeration. Fungal discs inoculated on coconut water gulaman at pH 6.0 were subjected to lighted and total dark conditions (to administer ample darkness, plates were covered with aluminum foil) to determine the illumination requirement. Finally, the optimal nutritional and physical factors previously established were used in evaluating the favorable temperature for mycelial growth. Plates were incubated by altering the temperature requirements: room temperature (32°C); air–conditioned (23°C); and refrigerated (9°C). Mycelial growth on agar plates as influenced by physical parameters was measured on the basis of mean radial mycelial growth and biomass for ten trials.

Mother spawn production

The mycelial growth performance of *G. lucidum* on various spawning materials like: corn grit, rice grain, sorghum seed and wood chips was also evaluated. The granular spawning materials were boiled, drained and maintained to 65% moisture content. 50 g of each substrate were dispensed in a sterile bottle, cotton–plugged, paper–wrapped and sterilized at 15 psi, 121°C for 45 minutes. Once cooled, 10 mm fungal disc was inoculated and incubated at room temperature allowing mycelial ramification. The number of days of total mycelial ramification of the substrates was recorded. The substrate with the thickest mycelia and fully ramified in the shortest period of time was chosen as the best spawning material.

Substrate formulation for basidiocarp formation

Corn grit as the most desirable grain spawn from the preceding evaluation was aseptically inoculated into sterilized fruiting bags (polypropylene) containing the different rice straw–sawdust based substrate formulations as follows T1: 100% rice straw, T2: 70% rice straw + 30% sawdust, T3: 50% rice straw + 50% sawdust, T4: 30% rice straw + 70% sawdust and T5: 100% sawdust. Fruiting bags were incubated at room temperature until the complete ramification of mycelia. The fully colonized bags were opened permitting primordia developed into mature basidiocarps. Fruiting bodies were harvested once the pilei became entirely red and the white edge vanished

(Chen 2002, Curvetto 2002, Gurung et al. 2012). The biological efficiency (BE) was calculated for each treatment from three flushes in a harvest period of 25 days (Royse 1990). BE = (fresh weight of harvested mushrooms) / (dry matter content of the substrate) x 100.

Morphogenesis

Basidiospores were collected from the mature basidiocarps by placing the fruiting body in a sterile Petri plate with filter paper. Spore suspension was prepared following the protocol of Karadeniz et al. (2013) with minor modification. The spores were bath in a test tube supplemented with 3% concentration of H_2O_2 for 10 seconds to prevent bacterial contamination and diluted with 10 mL of distilled water. Spore concentration of 1×10^8 CFU/mL in McFarland Nephelometer Standard was verified by the use of UV/VIS spectrophotometer (APEL–100, Japan).

One µL obtained from the spore suspension was inoculated onto coconut water gelatin adjusted to pH 6.0 plates. Plates were incubated at room temperature under continuous illumination allowing basidiospore germination. The initiation of spore germination was observed and documented under a stereo microscope every 24 hours. The developmental biology of the fruiting body was also documented every 24 hours using a digital camera.

Data were analyzed in the SPSS 16.0 computer software using analysis of variance (ANOVA) in one way classification system and Kruskal–Wallis test to determine the significant treatment comparison at 5% level of significance. Data with two treatments were analyzed using t–test.

Results and discussion

Influence of culture media

The mycelial growth performance and biomass of *G. lucidum* on five different indigenous culture media is shown in Table 1. The fast rate of mycelial growth was prominent in CWG obtaining the highest mean radial mycelial growth and biomass of 42.76 mm and 0.4725 g, respectively, which is significantly higher among all the treatments. Similarly, among the five indigenous culture media, a very dense and luxurious mycelial growth can be observed on plates with CWG. Even though the mycelia of *G. lucidum* spread rapidly on plates with CGDSG, RBDSG and SSDSG, their individual mycelial biomasses were extremely poor.

Table 1 Mycelial growth and biomass of *G. lucidum* on the different indigenous culture media.

Culture Media	Radial Mycelial Growth (mm)	Mycelial Biomass (g)
CWG	42.76 ± 1.44^a	0.4725 ± 0.01746^a
CGDSG	39.82 ± 2.04^b	0.2489 ± 0.02060^b
RBDSG	37.10 ± 1.04^c	0.2210 ± 0.01042^c
SSDSG	39.58 ± 1.44^b	0.2458 ± 0.01442^b
SPG	38.59 ± 1.85^{bc}	0.2433 ± 0.02076^{bc}

(In table: Values are the Mean ± SD. Means within a column having the same letter of superscript are insignificantly different from each other at 0.05 level of significance using Scheffe.)

The ability of the coconut water to stimulate rapid mycelial growth of *G. lucidum* is attributed to its nutritional content. Fresh coconut water is hygienic and nourishing. Being a fluid endosperm of the coconut fruit, it contains minerals as well as other essential nutrients for the induction of morphogenesis (i.e. induce fungal cells to divide and grow rapidly). In the Philippines, it was creatively used as a medium for the biotechnological production of schizophyllan from *Schizophyllum commune* and other edible mushrooms (Reyes et al. 2009). Since the Philippines is the major producer of coconut in the globe and a quarter (i.e. 3 million hectares) of its cultivated land is planted to coconut, coconut water is abundant. As soon as the coconut is cracked and its meat is utilized for oil production, its water becomes waste and oftentimes discarded. In the Philippines, housewives developed this renewable resource as fermentation substrate for the household production of *nata de coco* and vinegar. With its abundance and nutritional attributes

along with the outstanding performance of *G. lucidum* in CWG as reported in this paper, coconut water can be used as a suitable and economical medium in order to enhance the biomass in the cultivation of *G. lucidum*.

Influence of physical factors

pH

The optimal pH range for promoting mycelial growth of *Ganoderma* species has been reported to be 5.5 – 6.0 (Lee et al. 2008) comparable with the results of the recent study i.e. a pH of 6.0 for favorable mycelial growth of *G. lucidum* with a maximum average dry weight of 0.4740 g in 5 days. However, the mycelial growth and biomass of *G. lucidum* was nearly identical at pH 6.0 and 6.5 in this range (Table 2). Moreover, Lee et al. (2008) stated that Korean strains in *G. lucidum* grew over a wide pH range (i.e. 5.0–9.0) but efficient mycelial growth was found at pH 5.0. Jo et al (2009) specified pH 6.0–9.0 to be suitable for growth of *G. applanatum*. In addition, the maximum mycelial growth of *G. resinaceum* was obtained at an initial pH of 7.0 (Choi et al. 2006).

Mushrooms release enzymes that break organic matter down into an absorbable form for their vegetative structure. Enzyme activity is greatly affected by pH and mushroom species have evolved the means to function under their particular environment. In the present study, enzyme activity of *G. lucidum* was adversely affected by increasing the pH. When the medium is basic, the mycelium will shut down completely and die (Chang & Miles 2004). Thus, the basicity of the medium will denature the cells and render them functionless.

Aeration

The experiment demonstrated that parafilm sealing significantly influenced the rate of colonization as shown in Table 2. It can be seen that the higher radial mycelial growth was observed in sealed plates with a mean of 51.11 mm compared to those incubated in unsealed condition with a radial growth of 44.12 mm. This result suggests that aeration is another important physical factor to be considered for efficient mycelial growth of *G. lucidum*.

Table 2 Mycelial growth and biomass of *G. lucidum* as influenced by the different physical factors.

Physical factors	Radial Mycelial Growth (mm)	Mycelial Biomass (g)
pH		
pH 6.0	45.73 ± 1.03^a	0.4740 ± 0.01578^a
pH 6.5	44.45 ± 1.42^{ab}	0.4520 ± 0.01874^{ab}
pH 7.0	44.07 ± 1.23^b	0.4300 ± 0.02000^{bc}
pH 7.5	41.01 ± 0.56^c	0.4180 ± 0.00632^{cd}
pH 8.0	38.98 ± 0.83^d	0.4020 ± 0.02201^{cd}
Aeration		
Sealed	$51.11 \pm 1.65^*$	$0.4940 \pm 0.01075^*$
Unsealed	44.12 ± 1.40	0.4280 ± 0.01398
Illumination		
Lighted	$48.92 \pm 0.85^*$	$0.4890 \pm 0.01524^*$
Total Darkness	44.57 ± 1.47	0.3430 ± 0.01337
Temperature		
Air–conditioned (23°C)	12.00 ± 1.28^b	0.1287 ± 0.01736^b
Refrigerated (9°C)	02.62 ± 0.54^c	0.0462 ± 0.01291^c
Room Temp. (32°C)	50.67 ± 1.91^a	0.4980 ± 0.01317^a

(In table: Values are the Mean ± SD. Means within a column having the same letter of superscripts are insignificantly different from each other at 0.05 level of significance using Kruskal–Wallis. * significantly different at 0.05 level of significance using t-test.)

Illumination

The results on the evaluation of illumination as important physical factor are also presented in Table 2. Plates incubated in lighted conditions have a mean radial mycelial growth and biomass of 48.92 and 0.4890, respectively, which is significantly higher than plates incubated under complete

darkness. To put it briefly, the presence of light in combination with the carbon and nitrogen sources present in CWG serves as a signal for *G. lucidum* to be in flight, thus stimulated rapid mycelial growth in order to yield maximum biomass in the shortest period of 5 days. It has also been projected that mycelial growth in the presence of light promotes accumulation and the formation of fungal food reserves such as glycogen and lipids which are vital for generating macroscopic mushrooms from microscopic mycelium (Chang & Miles 2004). Furthermore, light as a fundamental abiotic factor excites sporulation (Druzhinina et al. 2008). However, as observed in this paper, light during mycelial growth also stimulated hyphal branching and conidiation of *G. lucidum* on CWG.

Temperature

Generally, the minimum and maximum cardinal temperatures for the mycelial growth and biomass of *G. lucidum* were 9°C and 32°C, respectively. Mycelia of *G. lucidum* had been expedited at room temperature (32°C) which was significantly higher than all of the treatments. In contrary, mycelial growth of *G. lucidum* appeared to be drastically suppressed at 9°C in proportion to the drop of temperature. This is caused by the denaturation and inactivation of essential enzymes which catalyze metabolic processes of *G. lucidum* (Lee et al. 2008). Optimum dry growth weight occurred at 32 °C with an average biomass of 50.67 g in 5 days (also shown in Table 2). Similarly, Lee et al. (2008) reported that the optimal temperature for the mycelial growth of Korean strains of *G. lucidum* was found at 30°C.

Evaluation of Spawn

Spawn quality is the key element in the production of medicinal mushrooms (Awi–waadu & Stanley 2010). To determine the mycelial growth of *G. lucidum* on the different granulated materials, this sub-study was conducted.

On the 5th day of incubation, the corn grits were fully ramified by robustly growing mycelia of the *G. lucidum* (Fig 1) whereas the sorghum seeds were fully ramified on the seventh day. The other substrates (i.e. rice grain and woodchips) were fully colonized on the ninth and fourteenth day of incubation, respectively. The results clearly proved that among various substrates used, maximum growth rate was observed in corn grits while the minimum mycelial extension was in woodchips. The second best granular spawning materials for *G. lucidum* were sorghum seeds.

The high carbohydrate, fatty acid, and protein component of corn grit could stimulate fruiting. Furthermore, Awi–waadu & Stanley (2010) found out that larger surface area and aperture of substrates improve spawn run which could account for the result obtained in corn grits. From the results recorded, basidiocarps of *G. lucidum* can be definitely cultured using agro–industrial residues in conjunction with locally available granular spawning materials.

At the latter part of the vegetative spawn run, mycelia began to form constricted growth in loops as response to ecological stimulus. Immediately, the production of basidiocarps of *G. lucidum* was evaluated on rice straw and sawdust from agro–industrial residues as base substrate.

Fig. 1 – Mycelial growth of *G. lucidum* on the different spawning materials namely, (A) corn grit, (B) rice grain, (C) sorghum seed and (D) woodchips after 5 days of incubation.
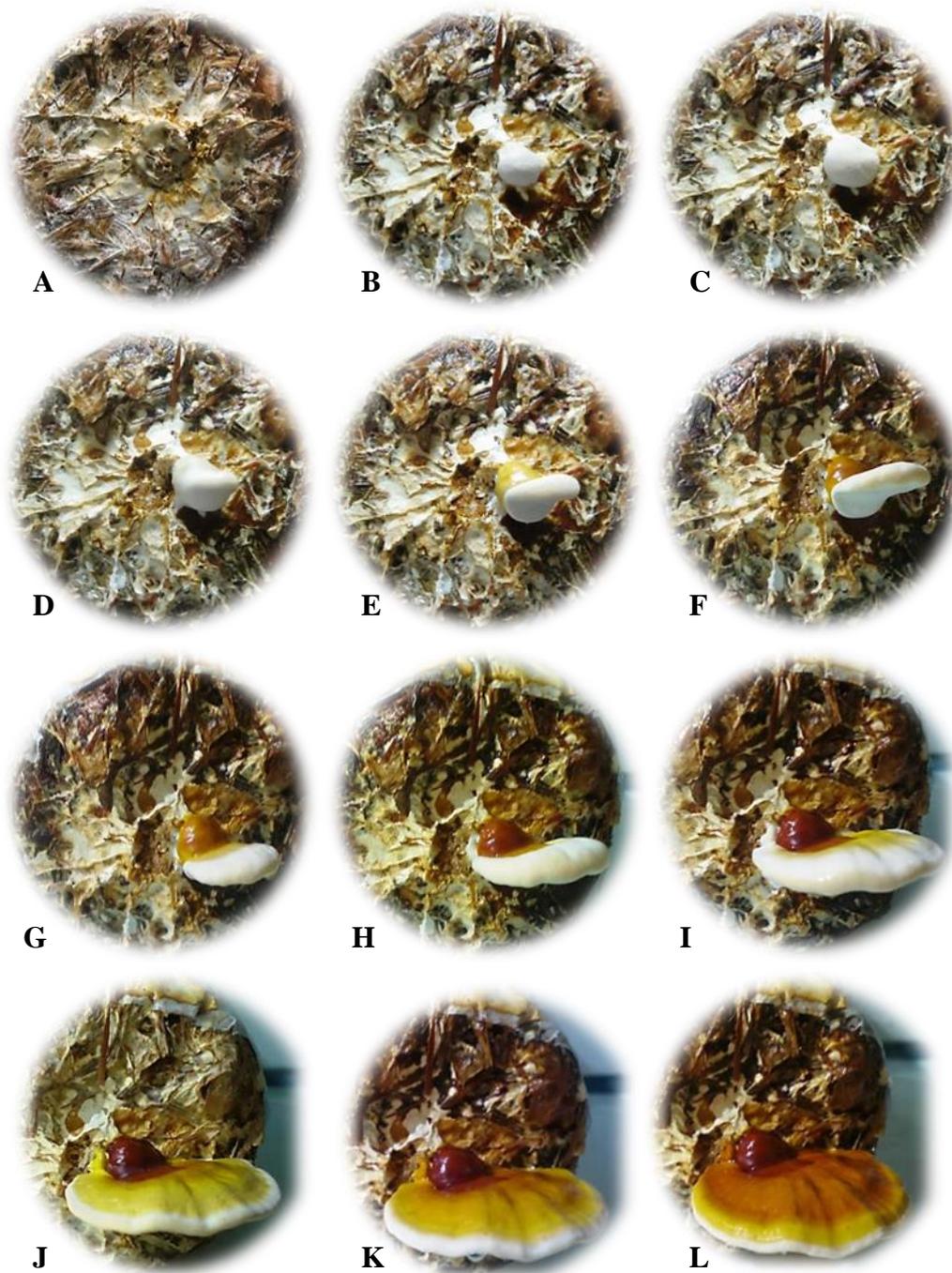
Fig. 2 – Morphogenesis of the basidiocarp of *G. lucidum.* (A-C) primodium development, (D-F) antler-like formation, (G-I) pileus expansion, and (J-L) bean-shaped and maturation stage.
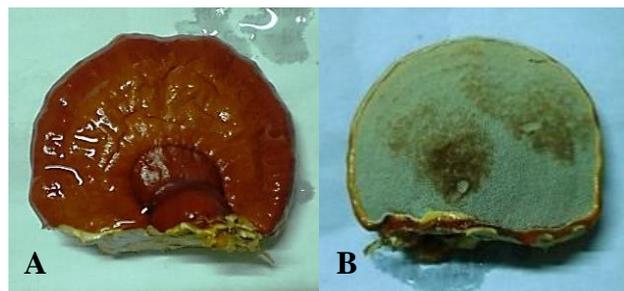
Fig. 3 – Mature spore shredding *G. lucidum* in (A) top and (B) bottom view.

Fruiting Development and Production

The growth and development of basidiocarp of *G. lucidum* is presented in Fig 2. The morphogenesis begins at the center of constricted mycelial growth which gradually developed into amorphous primordial mass and eventually into distinct primordium which surmounted the periphery of the substrate as whitish rounded mounds (Fig 2B). The primordium elongated perpendicularly into antler-like structure (Fig 2E). Under optimize cultural growth conditions, the antler-like basidiocarp began to expand horizontally giving rise to an undeveloped pileus (Fig 2G). The pileus then developed into a typical bean–shaped (Fig 2I). Temporarily, as the pileus became fully grown, yellowing of the pileus margin appeared (Fig 2J). Full maturity is then indicated when the pileus was fully maroon or the undifferentiated white growth at the edge of the basidiocarp disappeared (Fig 3A). Spores were already shed on the top of the pileus.

Less visible, but more essential, was the differentiation of a fertile layer called the hymenium underneath the mushroom cap (Fig 3B). The hymenium contained long fecund tubes (i.e. the end of the tubes perceived as pores with the naked eye) where basidiospores are produced. Thus, *G. lucidum* is woody polypore, fairly different from the fleshy oyster mushroom with gills and a centrally attached stalk. This information is fairly significant since a biomedically essential constituent (i.e. triterpenoid) is manufactured in this region (Chang & Miles 2004). Thus, this defines the medicinal value of the fruiting bodies *G. lucidum*.

The time intervals of the cultivation phases of *G. lucidum* are depicted in Table 3. Approximately, it will take 25 days to complete its cycle from primordial formation to fruiting body maturation (Chen et al. 2002). Substrates having 70% rice straw + 30% sawdust had the shortest period of 17.33 days for fructification. It was followed by 100% rice straw, which was statistically comparable to 50% rice straw + 50% sawdust, both had 20.00 days. Similarly, in terms of yield, 70% rice straw + 30% sawdust produced the heaviest weight (17.76 g), which statistically the same with 100% rice straw (17.40 g) and 50% rice straw + 50% sawdust (16.14 g).

Table 3 Fructification, yield and biological efficiency of *G. lucidum* on the rice straw − sawdust based substrate.

Substrate Formulations	Fructification (day)	Yield (g)	BE (%)
100% Rice Straw	20.00 ± 0.000^b	17.40 ± 0.629^a	6.011 ± 0.263^a
70% Rice Straw + 30% Sawdust	17.33 ± 4.619^c	17.76 ± 0.997^a	6.260 ± 0.435^a
50% Rice Straw + 50% Sawdust	20.00 ± 0.000^b	16.14 ± 0.170^a	5.663 ± 0.125^a
30% Rice Straw + 70% Sawdust	39.00 ± 0.000^a	13.62 ± 1.000^b	4.840 ± 0.345^b
100% Sawdust	37.33 ± 2.887^a	14.64 ± 2.365^b	5.252 ± 0.812^{ab}

(In table: Values are the Mean ± SD. Means within a column having the same letter of superscripts are insignificantly different from each other at 0.05 level of significance using Kruskal–Wallis.)

In the study of Erkel (2009), information on the various formulations of rice straw and sawdust in the cultivation of *G. lucidum* is insufficient. Hence, the results of the present study suggested that the yield and BE were significantly affected not only by pure sawdust but also by its combination with rice straw. Under the optimized growth parameters, the subsequent flushes have lower yield when compared with the results obtained by Chen et al. (2002). Thus, addition of some enrichment materials in the formulated rice straw − sawdust based substrates is highly recommended in the future studies to further improve the yield and BE of *G. lucidum*.

Process of Basidiospore Germination and Hyphal Growth

The germination of spores marks the beginning of the life cycle of a mushroom. Chaiyasut et al. (2010) found out that basidiospores of *G. lucidum* contain various biologically active substances which are more abundant than in the basidiocarps. However, sporoderm of *G. lucidum* comprises of calcium (19.01%), chitin (52.08% − 57.64%) and silica (19.01%) (Jungjing et al., 2007) which limits the absorption of these bioactive compounds.

CWG which was the best medium for mycelial growth under the optimized cultural conditions was used for germination since Ho & Nawawi (1986) recommended that factors favorable to mycelial growth of *Ganoderma* species were also favorable to the germination of their spores. Moreover, basidiospores can also germinate on nutrient medium to hyphal growth or by yeast–like budding (Smith et al. 2002).

Fig 4 presents the germination of the basidispore of *G. lucidum* every 24 hours of incubation. At 48 hours of incubation, spores were not yet germinated (Fig 4A) similar with the germination studies made by Chaiyasut et al. (2010). However, at 72 hours, the spores swelled and produced a short, initial hypha (a single germ tube) from the truncated end (Fig 4B). Early elongation commenced at 96 hours, while late elongation peaked at 120 hours. Septation of the germ tubes succeeded on the sixth day of incubation (Fig 2E) and complete breaking of the spores was found by seventh day. The massive branching was so profuse that mycelia in the course of monokaryotization were already formed around the germinated spores (Fig 4F). Anastomosis and clamp connections were also observed. In contrary, the results of the present investigation did not conform to those described by Dulay et a.l (2012a) on the local counterpart of shiitaki mushroom (*Lentinus tigrinus*).
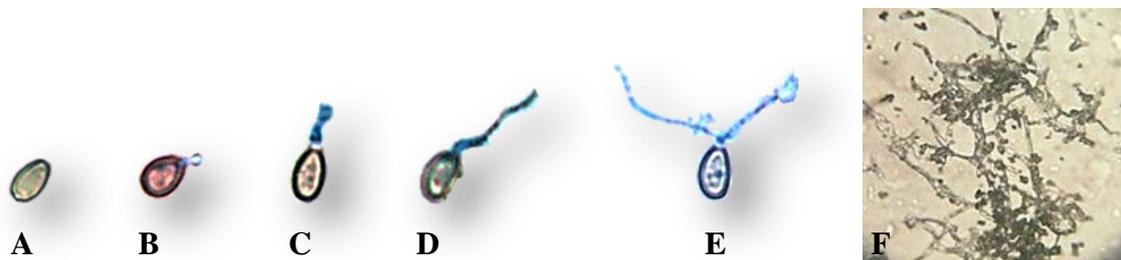
Fig. 4 – Process of basidiospore germination of *G. lucidum* after (A) 48 hours, (B) 72 hours, (C) 96 hours, (D) 120 hours, (E) 144 hours, and (F) 7 days of incubation.

With the significant findings obtained from the present study, it was established that a naturally occurring strain of *G. lucidum* can be cultured in locally available crops and in agro–industrial residues and maybe useful for its large scale production. Hence, the macrofungi reported in this paper is a promising contribution to the records of successfully domesticated wild nutraceutical mushrooms in the Philippines. Further studies on the use of other indigenous culture media in understanding the growth performance of *G. lucidum* are highly recommended. Furthermore, cultivation of other wild nutraceutical mushrooms on rice straw as basal medium enriched with other supplements is also recommended. Lastly, works on the threats on the incessant use of wild mycorural resources of the Philippines particularly in Nueva Vizcaya is also necessary.

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