Culture conditions for mycelial growth of eight Philippine wild strains of tiger sawgill mushroom, *Lentinus tigrinus*


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Abstract

*Lentinus tigrinus* is a wild edible and medicinal mushroom with potential culinary and pharmaceutical applications. In the present work, we evaluated the different culture media, pH and physical conditions such as aeration, illumination and temperature for maximum mycelial growth of eight *L. tigrinus* strains. Mycelial growth of the eight strains respond differently to the nutritional and physical factors. All strains showed maximum mycelial growth on coconut water gulaman (CWG), except TLLt 3, which favored potato dextrose agar (PDA) and malt extract agar (MEA). TLLt 1, 5, 6, 7 and 8 mycelia luxuriantly grew at pH 5.0-8.0 whereas TLLt 2 preferred pH 5.0-7.0, and TLLt 3 and 4 favored pH 5.0-6.5. Sealed condition was found to be more suitable for TLLt 1 mycelia while no significant difference was noted between the two aeration conditions for the mycelial growth of other seven strains. Both TLLt 1 and 2 mycelia favorably grew in lighted condition while the other six favorably grew in both lighted and dark conditions. Room temperature (30⁰C) recorded the fastest mycelial growth fo all strains, but found comparable to air-conditioned temperature (23⁰C) for the growth of TLLt 3, 6 and 8 mycelia. Therefore, mycelial growth of *L. tigrinus* on the different nutritional and physical parameters may vary depending on the strain type.

Keywords: *Lentinus tigrinus*, mycelia, commercial culture media, laboratory made media.

Introduction

*Lentinus tigrinus* is a culinary-medical mushroom which is popularly known as tiger sawgill mushroom or *kabuteng tigre* due to the appearance of the gills and presence of small brown scales on its basidiocarp. This mushroom is a white rot basidiomycete belonging to Class Agaricomycetes, Order Polyporales and Family Polyporaceae. It is characterized by leathery flesh and tough fruiting body and commonly grows singly or in group on fallen logs or rotten branches during the onset or middle part of rainy season (Dulay et al., 2012). This mushroom was recently, observed growing in various parts of Luzon, Philippines.

As a culinary food ingredient, the basidiocarp contains proteins, carbohydrates, ash and fibers but low in fat and energy value (Dulay et al., 2014; Pourianfar et al., 2020). As a
medicine, the extracts contain bioactive compounds that exhibit a number of medicinal attributes such as antioxidant, antibacterial, antifungal (Sevindik, 2018; Dulay et al., 2017), antiproliferative and pro-apoptotic (Mohammadnejad et al., 2019) and hypoglycemic activities (Dulay et al., 2014). Gao et al. (2019) identified fungal proteins (Fip-lti1 and Fip-lti2) from L. tigrinus that can protect the liver from Con A induced liver oxidative injury. The dichloromethane extract contains cerevisterol, stellasterol and ergosterol which have been reported to exhibit medicinal activities such as anti-inflammatory, antibacterial and antioxidant activities in other mushroom species (Ragasa et al., 2018). Laccase with inhibitory activity against HIV 1 reverse transcriptase was isolated from the broth of mycelial culture (Xu et al., 2012). The occurrence of mushrooms in the wild is seasonal so that most of them appear only during rainy season. Moreover, when they appear, the amount is not sufficient. Optimization of culture conditions and domestication of wild mushrooms are necessary for the continuous supply fruiting bodies or mycelia for evaluation of their biological activities. In our desire to search for additional strains of L. tigrinus which can be used as source of nutritious food and to develop drugs and functional foods, we evaluated the nutritional and physical requirements for mycelial growth. Therefore, the present study established the suitable culture media and physical conditions for mycelial growth, maintenance of germplasm and cultivation of different strains of L. tigrinus.

Materials and Methods

Tissue Culture

Fruiting bodies of eight strains of L. tigrinus were collected from the different areas of Luzon Island, Philippines. Small tissue from the inner part of the fruiting body was aseptically cut using sterile scalpel and inoculated into a sterile PDA slant. The tissue was allowed to colonize the medium for one week. Afterward, mycelial block was transferred into PDA plates and allowed to grow for a week. The agar plate culture served as the source of inoculum for the evaluation of nutritional and physical requirements for mycelial growth of the different strains.

Evaluation of culture media

To assess the nutritional requirements of different strains of L. tigrinus, various culture media were used i.e. four commercial culture media and four home-made culture media. The commercial media include potato dextrose agar (PDA), malt extract agar (MEA), mycological agar (MA) and sabouraud dextrose agar (SDA). These media were prepared according to the manufacturer’s instruction written in the label. On the other hand, the home-made culture media namely: potato sucrose gulaman (PSG), coconut water gulaman (CWG), corn grit decoction gulaman (CGDG) and rice bran decoction gulaman (RBDG) were prepared following the preparations described by Kalaw et al. (2016) and Dulay et al. (2012). All culture media were sterilized in an autoclave at 121°C, 15 psi for 30 min. Once cooled, approximately 20 ml of the media were dispensed in previously sterilized petri plates and allowed to solidify overnight. Mycelial disc (10 mm diameter) from a week-old L. tigrinus culture was inoculated at the center of the plate. The mycelial growth rate was determined and the mycelial density was also observed. Each treatment was replicated thrice.

Evaluation of pH

The pH requirement was assessed using the most suitable culture medium. The pH of the medium was adjusted at 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 using 0.1 M hydrochloric acid and 0.1 M sodium hydroxide. Afterwards, the media were autoclaved at 121°C, 15 psi for 30 min. Media were pour-plated and allowed to cool. Mycelial disc (10 mm diameter) from a week-old culture was inoculated at the center of the plate. The mycelial growth was measured at 7th day incubation. The mycelial density was visually assessed. The experiment was done in triplicate.

Evaluation of physical factors

Aside from nutritional factor, the influence of aeration, illumination and temperature was also determined. The protocol outlined by Kalaw et al. (2016) was used in this study. In the determination of the most appropriate aeration condition, the first set of plates was sealed with parafilm while the second set of plates were not sealed with parafilm. In illumination experiment, culture plates were wrapped with black cloth while the other plates were illuminated with fluorescent
light. Finally, to determine the influence of temperature, the culture plates were incubated in three temperature conditions namely: room temperature (30°C), air-condition temperature (23°C) and refrigeration temperature (9°C). All treatments were prepared in triplicate.

Results and Discussion

Effect of culture media
In vitro cultivation of mushroom mycelia requires a suitable culture medium that will support efficient growth and development. This culture medium contains the essential nutrients needed by the mushroom organism. Several commercial and laboratory made culture media are now being used as culture medium for the maintenance of the pure culture of mushroom. The influence of four commercially available dehydrated culture media and four indigenous culture media on the mycelial growth of eight L. tigrinus strains was evaluated. Table 1 shows the mycelial growth rates of eight strains as affected by the different culture media. It can be seen that each strain of L. tigrinus respond differently to the eight culture media. All strains showed the highest mycelial growth rate on CWG, except TLLt 3 which favored PDA and MEA. Aside from CWG, TLLt 2 mycelia also favored SDA, whereas TLLt 5 and 7 also preferred RBDG and TLLt 4 also favored RBDG, MEA and PDA, which showed no significant difference with CWG. These results suggest that each strain has specific culture medium preference.

It was found that among eight strains, mycelia of seven strains exceedingly favored CWG. The superiority of CWG could be attributed to its nutrient compositions. Coconut water contains sugars, inorganic ions, vitamins, minerals, amino acids, enzymes, auxin, cytokinin, diphenylurea and trace elements such as zinc, selenium, iodine, sulfur, manganese, boron, molybdenum etc. (Yong et al., 2009; Prades et al., 2012). Earlier studies also reported that coconut water is an ideal medium for mycelial growth Pleurotus citrinopileatus, Pleurotus djamour, Pleurotus salmonoeostreaminus and Ganoderma lucidum (Jacob et al., 2015; Magday et al., 2014; Zurbano et al., 2017). On the other hand, commercially-available media such as PDA and MEA were also found suitable for one strain. Similarly, Agrocybe aegerita, Pleurotus ostreatus, and Volvariella volvacea exhibited faster mycelial growth on MEA (Muthu and Shanmugasundaram, 2015; Nasim et al., 2001).

Statistical analysis
The data gathered were statistically analyzed using SPSS version 16. Turkeys HSD was used in comparing more than two means while T - test was employed in comparing means of experiments with two treatments at 5% level of significance.

In addition, Kumar et al. (2020) observed that optimum mycelial growth of Pleurotus ostreatus was obtained in PDA.

Effect of pH
The pH of the medium is an important factor that can influence the rate of mycelial growth and proliferation. Table 1 presents the pH requirement of eight strains of L. tigrinus. It is interesting to note that all strains can grow from pH 5.0 to 8.0. Strains TLLt 1, 5, 6, 7 and 8 can grow on a wide range of pH as indicated by the no significant differences in mycelial growth in all pH levels evaluated. However, the other three strains preferred narrower pH range. TLLt 2 required 5.0 to 7.0, whereas TLLt 3 and 4 favored pH between 5 - 6.5 and 5 - 6.0, respectively. All strains showed very thick mycelia except TLLt 1 and 8, which do not possess thick mycelia. The data obtained in the present study imply that the pH requirement of mushroom is not only species specific but also strain dependent. In a similar work, Kalaw et al. (2016) reported difference in pH requirements of two L. tigrinus strains, CLSU strain A and CLSU strain B which displayed larger mycelial growth at pH 6 to 6.5 and 6, respectively. On the other hand, Dulay et al. (2012) disclosed that the optimum pH of L. tigrinus was found at 7 to 8. Abon et al. (2020) reported that the optimum pH for Volvariella volvacea La Clementina strain and Vinces strains was at 6.5 to 7.5 and 6.5 to 7 for Montalvo strain.

Influence of aeration
Oxygen affects cell growth, cellular morphology, nutrient uptake and metabolite biosynthesis (Simonic et al., 2008). The aeration requirements of L. tigrinus strains were determined by incubating them in sealed and unsealed conditions. It can be noticed that aeration did not influence the mycelial growth of seven strains of L. tigrinus as indicated by no significant difference in mycelial growth between sealed and unsealed conditions (Table 2). However, L. tigrinus TLLt 1 strain showed faster mycelial growth in sealed condition. In terms of mycelial density, all strains displayed very thick mycelia in both conditions except
TLLt 8, which do not exhibit thick mycelia. Results suggest that aeration was not a major physical factor in most L. tigrinus strains, although it is in some strains. The findings corroborate early reports regarding the influence of aeration on mushrooms. For instance, Abon et al. (2020) reported that the three Ecuador strains of Volvariella volvacea were not significantly influenced by aeration conditions. Same with the mycelial growth of Cyclocybe cyndracea and P. djamour (Landingin et al., 2021; Bumanlag et al., 2018. In fact, Reyes et al. (1998) found out that V. volvacea preferred sealed conditions.

**Influence of illumination**

Light conditions during mycelial growth are known to influence fungi in many ways (Rangel et al., 2011). Although not photosynthetic, mushrooms need light to stimulate the mycelial growth. The influence of light was assessed by incubating the culture plates in lighted and dark conditions. Apparently, TLLt 1 and 2 strains significantly displayed faster mycelial growth and very thick mycelia in lighted condition, whereas the mycelial growth of other six strains showed no significant different between lighted and dark conditions (Table 2). Both conditions also showed very thick mycelial growth of the six strains. These results suggest that the strains evaluated exhibited varied responses to illumination. Variations in mycelial growth response of different species and strains of mushrooms in light and dark conditions have been documented in earlier reports. For example, Abon et al. (2020) found out that the mycelia of two strains of Ecuador Volvariella volvacea favorably grew in lighted condition while one strain exceedingly favored dark conditions. Similarly, Quiaco et al. (2014) observed that light was found not essential for mycelial growth of three strains of Lentinula edodes. On the other hand, the mycelial growth of Auricularia polytricha and Auricularia auricula is better in artificial light compared to darkness (Priya and Geetha, 2016). Lastly, Dulay et al. (2021) disclosed that the mycelia of Trametes elegans was not influenced by lighted and dark conditions.

**Influence of temperature**

Among the different physical parameter, temperature is considered as the most fundamental factor. It determines the distribution of fungi (Hudson, 1986). The influence of temperature was also determined by incubating the culture plates in three temperature conditions such as room temperature (30°C), air-condition temperature (23°C) and refrigeration temperature (6°C). It can be seen that TLLt 1, 2, 4, 5 and 7 significantly recorded higher mycelial growth when incubated at 30°C (Table 2). However, mycelial growth of TLLt 3, 6 and 8 showed no significant difference between 30°C and 23°C, suggesting that both temperature conditions are favorable for growth. Contrastingly, no mycelial growth of all strains was observed at 6°C. The results imply that the temperature requirement for mycelial growth of L. tigrinus is strain dependent and the strains evaluated are suitable to the tropical conditions of the country. The present study established that the eight strains of L. tigrinus are considered tropical mushrooms as they favorably grew at 30°C. This agrees with the observation of Kerketta et al. (2017) that the mycelial growth of each strain Calocybe indica significantly varied in all temperature evaluated. However, Alam and Rahman (2020) reported that the 25°C was the optimum temperature for mycelial growth of five strains of Pleurotus salmoneostrominins.

Table 1. Effect of culture media and pH on the mycelial growth of the eight strains of Lentinus tigrinus.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Mycelial growth rate (day⁻¹)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TLLt 1</td>
</tr>
<tr>
<td><strong>Media</strong></td>
<td></td>
</tr>
<tr>
<td>CWG</td>
<td>15.00a</td>
</tr>
<tr>
<td>PSG</td>
<td>12.59ab</td>
</tr>
<tr>
<td>CGDG</td>
<td>11.78c</td>
</tr>
<tr>
<td>RBDG</td>
<td>12.05b</td>
</tr>
<tr>
<td>PDA</td>
<td>11.61c</td>
</tr>
<tr>
<td>MEA</td>
<td>7.47a</td>
</tr>
<tr>
<td>SDA</td>
<td>12.82ab</td>
</tr>
<tr>
<td>MA</td>
<td>7.26a</td>
</tr>
</tbody>
</table>

Means with similar superscripts are statistically comparable from each other using Tukey’s HSD at 5% level of significance. CWG, coconut water gulaman; PSG, potato sucrose gulaman; CGDG, corn grit decoction gulaman; RBDG, rice bran decoction gulaman; PDA, potato dextrose agar; MEA, malt extract agar; SDA, Sabouraud dextrose agar; MA, mycological agar.

Table 2. Effect of physical factors on the mycelial growth of the eight strains of Lentinus tigrinus.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Mycelial growth rate (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TLLt 1</td>
</tr>
<tr>
<td><strong>Aeration</strong></td>
<td></td>
</tr>
<tr>
<td>Sealed</td>
<td>15.00a</td>
</tr>
<tr>
<td>Unsealed</td>
<td>14.25b</td>
</tr>
<tr>
<td><strong>Illumination</strong></td>
<td></td>
</tr>
<tr>
<td>Lighted</td>
<td>15.00a</td>
</tr>
<tr>
<td>Dark</td>
<td>13.76b</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td></td>
</tr>
<tr>
<td>9°C</td>
<td>ng</td>
</tr>
<tr>
<td>23°C</td>
<td>13.76b</td>
</tr>
<tr>
<td>30°C</td>
<td>15.00a</td>
</tr>
</tbody>
</table>

Means with similar superscripts are statistically comparable from each other using Tukey’s HSD and t-test at 5% level of significance. ng, no growth.

Conclusion

Each strain has unique nutritional and physical condition requirements for efficient mycelial growth even belonging to the same species. CWG at pH 5.0-8.0 incubated in either sealed or unsealed, either lighted or dark, and at 30°C are found to be the most suitable nutritional and physical conditions for the maximum mycelial growth of most L. tigrinus strains evaluated in this study. These information are very vital in maintenance of the germplasms of L. tigrinus in the culture collection laboratory and in the generation of production technologies for medicinal and industrial purposes. Evaluation of fruiting body production is currently under investigation in the laboratory.

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