
Cultivation of the edible mushroom Auricularia polytricha using sawdust-based substrate made of three Indonesian commercial plantation species, Falcataria moluccana, Shorea sp., and Tectona grandis


Colegio de Postgraduados
Puebla, México

Available in: http://www.redalyc.org/articulo.oa?id=68524018001
CULTIVATION OF THE EDIBLE MUSHROOM

Auricularia polytrichia using sawdust-based substrate made of three Indonesian commercial plantation species, Falcataaria moluccana, Shorea sp., and Tectona grandis*

D. Irawati1,2,3, C. Hayashi2, Y. Takashima1,2, S. Wedatama2, F. Ishiguri2, K. Iizuka2, N. Yoshizawa2 and S. Yokota2**

1 United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan.
2 Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan.
3 Faculty of Forestry, Gadjah Mada University, Yogyakarta, Indonesia.

Accepted for publication July 13, 2012

ABSTRACT

Auricularia polytrichia is an edible mushroom, also known as black jelly. In Indonesia, although A. polytrichia has been extensively cultured on the wood meal substrates, information is very limited on effects of wood meal from various tree species used for cultivation substrates on A. polytrichia cultivation characteristic. An investigation, therefore, is needed to find suitable tree species for the substrate of A. polytrichia cultivation. In this study, wood meals of 3 tropical hardwood species (Falcataaria moluccana, Shorea sp., and Tectona grandis) from Indonesia were used as basal cultivation substrates. The fastest mycelia growth was found in the substrate made of Shorea sp., and the highest glucosamine content was found in the substrates made

* Part of this work was presented at the 7th International Conference on Mushroom Biology and Mushroom Products, in Arcachon, France, October 2011.
** Corresponding author: S. Yokota, Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan. Tel.: +81-28-649-5539; Fax: +81-28-649-5545. E-mail: yokotas@cc.utsunomiya-u.ac.jp
of Shorea sp. and F. moluccana. No significant difference in the period of time to the first harvest was found between F. moluccana (23 days) and Shorea sp. (25 days), whereas a significant difference was found in the interval between the following harvesting periods (7 and 10 days for substrates made of F. moluccana and Shorea sp., respectively). Over the entire cultivation period, the substrates made of F. moluccana produced the highest fruiting body yield, greatest biological conversion, and greatest weight loss from the substrate. These results indicate that F. moluccana wood meal is the appropriate basal substrate for A. polytricha cultivation.

Key words: Auricularia polytricha, Falcataria moluccana, mushroom cultivation, Shorea sp., Tectona grandis.

INTRODUCTION

Auricularia polytricha is an edible mushroom, also known as black jelly. This mushroom is cultivated in tropical regions because its mycelium can grow at temperatures ranging from 10 to 40°C. Unlike other mushroom species, cultivation of A. polytricha is easy and fast to yield fruiting bodies. It does not require expensive facilities. In addition, various forestry and agricultural wastes have been used as substrates for cultivation of this mushroom. A. polytricha, therefore, has been extensively cultured in Indonesia using substrates made from various types of lignocellulosic materials12.

Indonesia is one of the tropical countries which have many forestry sites and highly active wood industries. According to statistical data from the Forestry Department of Indonesia, the total Indonesia log production in 2010 was 42.4 million cubic meter9. The amount of wood waste derived from forestry and wood industry was estimated to be 30-40%. Thus, utilization of wood waste is important in Indonesia. Using wood waste as cultivation substrates for A. polytricha is an effective utilizing method. The wood waste, however, comes from many different tree species. Mycelial growth and fruiting body formation are greatly affected by tree species and quality11. However, information is very limited about effects of wood meal from various tree species used as cultivation substrates. It is necessary, therefore, to investigate which tree species yields optimal mycelial growth and fruiting body productivity in A. polytricha cultivation.

In the present study, suitability of the wood from three commercial Indonesian plantation species, Falcataria moluccana, Shorea sp., and Tectona grandis, which are the main species providing high quality wood in Indonesia, for sawdust-based cultivation of A. polytricha was evaluated.

MATERIALS AND METHODS

Fungus and substrate materials. Auricularia polytricha (Mont.) Sacc. (Aragekikurage 89) was obtained from Mori & Company, Ltd., Japan. Wood meal (9–80 mesh) of 3 tropical hardwood species [Falcataria moluccana (Miq.) Barbeny & J.W. Grimes, Shorea sp., and
Tectona grandis L.f.] from Indonesia was used as the basal cultivation substrates. Commercial rice bran (Satoh Rice, Japan; 9–80 mesh size) was used as a nutritive additive. Wood meal [moisture content (MC)= 8.5–11.0%] and commercial rice bran (MC= 11.0%) were mixed in a weight ratio of 8:1. MC of wood meal and rice bran was determined by using an infrared moisture balance (FD-600; Kett Electric Laboratory, Japan). Calcium carbonate was added at a concentration of 6% (w/w) to adjust the pH of the cultivation substrate to between 6 and 7. MC of the substrate was adjusted to 70% by adding tap water.

Mycelial growth rate. To measure the mycelial growth rate, 20 g of cultivation substrate (MC= 70%) was packed in a Petri dish (90 mm diameter). Eight replications were performed for each wood meal substrate. The substrates were subsequently autoclaved at 121 C for 20 min, and inoculated with mycelial plugs (5 mm diameter) of A. polytricha, previously grown on potato dextrose agar (Difco, Bacto Dickinson and Company, U.S.A.) medium. The culture was maintained at 25 C in the dark. Colony diameter was measured every 3 days in four directions until the mycelia reached the edge of each Petri dish.

Glucosamine content. To determine the glucosamine content in the mycelium, 5 g of cultivation substrate was packed in a Petri dish (45 mm diameter). Chitin content in the mycelium was assayed by the method of Braid and Line⁰ and 3 repetitions were performed for each sample. To degrade fungal chitin into N-acetyl glucosamine, 1 g of the dry sample after 50 days of culture was hydrolyzed with 5 ml of 5 M HCl at 80 C for 20 h. Samples were then filtered through Whatman No. 41 filter papers (Whatman International, England), and a 1-ml aliquot was diluted with 5 ml of distilled water. This solution was subsequently added to a glass column (12.5 cm length × 1.5 cm diameter) containing the strong acidic cation-exchange resin Dowex 50W-X8 (60–80 mesh; Acros organics, U.S.A.), and glucosamine was eluted with 5 ml of 2 M HCl. One drop of 0.1% methyl red indicator was added to each tube, followed by adding drops of 50% KOH and 35% HCl until the red color reappeared. A 1-ml aliquot was transferred into a 20-ml test tube, followed by sequential addition of 1 ml 4.8% KHSO₄, 1 ml 4.8% NaNO₃, 1 ml 11.1% NH₄OSO₂NH₂, 1 ml 0.5% 3-methyl-2-benzothiazolone hydrazone hydrochloride, and 1 ml 0.5% FeCl₃ aqueous solution. The absorbance of the characteristic bluish green color was measured at 630 nm using a spectrophotometer (V-650; Jasco, Japan). The calibration curve was drawn with N-Acetyl-D-(+)-glucosamine (Wako Pure Chemical Co., Japan) as a standard at concentrations of 0, 10, 20, and 30 mg/ml.

Chemical components of the cultivation substrates. Chemical components of the substrates were determined before and after cultivation. Extraction with organic solvents was performed to determine the quantities of holocellulose, α-cellulose, Klason lignin, and ash. Before chemical analysis, the samples were ground with a rotary speed mill (P-14; Fritsch, Germany) and then sieved through 40–80 mesh size. Subsequently, the samples were dried in an oven at 45 C. Extraction with organic solvents was performed as follows: 5 g of sample was extracted with 120 ml of a mixture of ethanol and toluene (1:2, v:v) by a Soxhlet extractor for 6 h. The amounts of Klason lignin, holocellulose, and α-cellulose were determined by ordinary methods²,¹³. To determine the ash content, a 1 g sample was heated in a muffle furnace.
(FO 100; Yamato, Japan) at 600°C for 2 h, and then, after cooling in a desiccator, it was reheated at 600°C for 1 h. For all chemical component analyses, 3 replications were performed for each sample.

Fruiting body production. The cultivation substrate (150 g; MC= 70%) was packed in a polypropylene bag (25 × 8 × 4.5 cm) equipped with a porous sterile filter (1 cm diameter; MilliSeal, Millipore, U.S.A.) and then autoclaved at 121°C for 20 min. Twelve packs were prepared for each wood meal substrate. After inoculation with A. polytricha mycelia, the substrates were cultured for 50 days in a culture room at 20–23°C with a relative humidity of 70–80% in the dark. A flushing treatment was conducted by diagonally cutting one side of the polypropylene bag surface, and the cultivation substrates were further cultured for 80 days in a culture room at 25°C with a relative humidity of 80–90% under illumination by cool white color fluorescent tubes (179 lux, 24 h/day). To prevent the substrate’s surface from drying in culture room, water was sprayed to substrates everyday in order to keep them wet, i.e. around 70% MC. Fruiting bodies about 5 cm in diameter were collected, and their fresh and dry weights were measured. Fruiting bodies were collected 8 times from the substrates made of F. moluccana and Shorea sp., and 4 times from the substrate made of T. grandis.

Biological conversion. In some reports, biological conversion (BC) was calculated from fresh weight of fruiting bodies dividing by dry weight of substrates. However, fresh weight of A. polytricha fruiting bodies was largely affected by moisture content of fruiting bodies. Thus, in the present study, BC was calculated considering dry weight of fruiting bodies, according to the following equation:\[ \text{BC} (\%) = \left( \frac{\text{Weight of dry fruiting bodies harvested}}{\text{Initial weight of dry substrates}} \right) \times 100. \]

Substrate weight loss. The dry weight of the substrate before and after cultivation was measured to calculate the amount of weight loss in the substrate. Twelve replications were performed for each wood meal.

RESULTS AND DISCUSSION

The growth of A. polytricha mycelia on substrates made of 3 different tree species are shown in Fig. 1. Significant differences were found in the mean growth of mycelia on the three different substrates. In the sawdust substrate made of Shorea sp., the mycelium showed the fastest growth and reached the edge of the Petri dish 14 days after inoculation. On the other hand, the slowest growth was found in the substrate made of F. moluccana. The fast growth on the Shorea sp. substrate may be because of the lowest ethanol-toluene content in the pre-cultivation substrate (3.1%) [Table 1]. Wood extracts inhibit the mycelial growth of several mushrooms.

An abundant and thick mycelial growth was observed on the substrate made of F. moluccana, irrespective of the slower mycelial growth. The quantity of mycelia can be estimated by measuring the glucosamine content. Glucosamine content in samples incubated for 50 days was measured to evaluate the quantity of mycelia just before the flushing treatment. Fig. 2 shows the glucosamine contents of mycelia growing on the sawdust-based substrates. Among the 3 substrates used in the present study, the substrates made of F. moluccana and Shorea sp. produced the highest quantity of mycelia, and the values were statistically different from that of the substrate made of T. grandis,

indicating that *A. polytricha* does not grow well in the substrate made of *T. grandis*. This result may be explained by the high levels of ethanol–toluene extracts in *T. grandis* pre-cultivation substrate (7.2%) [Table 1]. The extracts of *T. grandis* wood contain desoxylpachol, palmitic acid, lapachol, tectoquinone, 2-hydroxymethyl anthraquinone, squalene, and tectol.

Table 1 shows the chemical components in the different substrates made of the 3 tree species, before and after cultivation. Most chemical components decreased following cultivation. The α-cellulose to lignin ratio in substrate made of *F. moluccana* was higher after cultivation, while it was slightly lower in substrates made of *Shorea* sp. and *T. grandis*. These results indicate that *A. polytricha* used the chemical components of the wood, especially α-cellulose and lignin, for mycelial growth and to produce fruiting bodies. A similar result was also found in substrates made of *Grifola frondosa*, which degraded lignin in sawdust substrates during cultivation.

Table 2 shows the period required for harvesting fruiting bodies, yield of fruiting bodies, biological conversion, and weight loss of substrate after cultivation. The period of time to the first harvest was shorter in the substrates made of *F. moluccana* (23 days) and *Shorea* sp. (25 days) than in that made of *T. grandis* (31 days). No significant difference was found in the period of time.
Table 1. Chemical components (%) and pH of wood meal substrates before and after cultivation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>E</th>
<th>H</th>
<th>C</th>
<th>L</th>
<th>C : L</th>
<th>A</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Substrate before cultivation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. moluccana</em></td>
<td>5.2 ± 0.2</td>
<td>73.4 ± 2.5</td>
<td>40.0 ± 0.2</td>
<td>19.4 ± 0.5</td>
<td>2.1</td>
<td>11.3 ± 0.4</td>
<td>7.8</td>
</tr>
<tr>
<td><em>Shorea sp.</em></td>
<td>3.1 ± 0.2</td>
<td>78.7 ± 0.3</td>
<td>54.8 ± 0.8</td>
<td>27.9 ± 0.3</td>
<td>2.0</td>
<td>6.9 ± 0.7</td>
<td>8.1</td>
</tr>
<tr>
<td><em>T. grandis</em></td>
<td>7.2 ± 0.4</td>
<td>73.3 ± 1.0</td>
<td>45.0 ± 0.9</td>
<td>27.5 ± 1.1</td>
<td>1.6</td>
<td>10.1 ± 0.6</td>
<td>7.8</td>
</tr>
<tr>
<td><strong>Substrate after cultivation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. moluccana</em></td>
<td>2.9 ± 0.3</td>
<td>41.0 ± 4.2</td>
<td>16.9 ± 0.8</td>
<td>5.5 ± 0.1</td>
<td>3.1</td>
<td>6.5 ± 1.4</td>
<td>4.8</td>
</tr>
<tr>
<td><em>Shorea sp.</em></td>
<td>2.2 ± 0.2</td>
<td>48.3 ± 0.7</td>
<td>23.1 ± 1.4</td>
<td>13.5 ± 1.6</td>
<td>1.7</td>
<td>5.3 ± 1.1</td>
<td>5</td>
</tr>
<tr>
<td><em>T. grandis</em></td>
<td>4.5 ± 0.4</td>
<td>62.0 ± 1.3</td>
<td>30.7 ± 0.4</td>
<td>21.9 ± 1.9</td>
<td>1.4</td>
<td>7.5 ± 1.2</td>
<td>5.8</td>
</tr>
</tbody>
</table>


to first harvest between the substrates made of *F. moluccana* and *Shorea* sp. However, a significant difference was found in the interval periods between the following harvests: 7 days for the substrate made of *F. moluccana* and 10 days for that made of *Shorea* sp. At the end of the cultivation period (80 days), the *F. moluccana* substrate gave the highest yield of fruiting bodies; total fresh weight, total dry weight, and number of fruiting bodies were 65.4 g, 7.6 g, and 11, respectively. The ratio of the dry weight of each fruiting body to the number of fruiting bodies showed the highest value (0.69 g) in the substrate made of *F. moluccana*. This result indicates that fruiting bodies from the substrate made of *F. moluccana* had the highest density. The highest fruiting body yield in the substrate made of *F. moluccana* may be related with the higher amount of mycelium in that substrate (Fig. 2). Ohga\textsuperscript{11} also reported that a higher fruiting body yield was obtained from those substrates which had high glucosamine contents in the cultivation of *Pleurotus abalonus* and *P. eryngii*. Thus, fruiting body yield could be evaluated by the amount of mycelium grown during the cultivation period.

The *F. moluccana* substrate showed the highest biological conversion and weight loss, 15.6% and 43.6%, respectively. Weight loss was also correlated in these experiments with the decrease in holocellulose (r= 0.95) and lignin (r= 0.89) contents, but not with ethanol–toluene extracts (r= -0.30) and α-cellulose (r= 0.65) contents (Fig. 3). This result supports those described previously that *A. polytricha* actively degraded holocellulose and lignin during mycelial growth and fruiting body formation. In general, chemical components contributing to large weight loss are incorporated into the fruiting bodies and partly emitted into the atmosphere as carbon dioxide through mushroom respiration\textsuperscript{16}. Furthermore, the weight loss is proportional to the fruiting body yield.
Table 2. Period required for harvesting *Auricularia polytricha*, yield, biological conversion, and weight lost of substrate. Tree species studied: *Falcataria moluccana*, *Shorea* sp., and *Tectona grandis*.

<table>
<thead>
<tr>
<th>Tree species</th>
<th>Period required for harvesting fruiting body (day)</th>
<th>Yield of fruiting body</th>
<th>DWM (g)</th>
<th>BC (%)</th>
<th>WL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st harvest</td>
<td>Between following harvests</td>
<td>FW (g)</td>
<td>DW (g)</td>
<td>N</td>
</tr>
<tr>
<td><em>F. moluccana</em></td>
<td>23 ± 5a</td>
<td>7 ± 1a</td>
<td>65.4 ± 4.5a</td>
<td>7.6 ± 0.8a</td>
<td>11 ± 1a</td>
</tr>
<tr>
<td><em>Shorea</em> sp.</td>
<td>25 ± 4a</td>
<td>10 ± 2b</td>
<td>52.6 ± 15.0b</td>
<td>5.2 ± 1.0b</td>
<td>8 ± 2b</td>
</tr>
<tr>
<td><em>T. grandis</em></td>
<td>31 ± 7b</td>
<td>13 ± 3c</td>
<td>32.0 ± 11.3c</td>
<td>2.9 ± 1.0c</td>
<td>5 ± 2c</td>
</tr>
</tbody>
</table>

FW= Fresh weight. DW= Dry weight. N= Number of fruiting bodies. DW:N= Ratio of dry weight to number of fruiting body. DWM= Dry weight of substrates after cultivation. BC= Biological conversion. WL= Weight loss of substrate. ± = Standard deviation. The same superscript letter followed by the average value shows no significant difference among the three tree species by Tukey-Kramer test at the 5% level. n = 12.

Fig. 2. Glucosamine content of *Auricularia* mycelia on the sawdust based substrates after 50-day incubation. Tree species studied: *Falcataria moluccana*, *Shorea* sp., and *Tectona grandis*. The same alphabet letter followed by a bar of standard deviation shows no significant difference among the three tree species by Tukey-Kramer test at the 5% level. n = 3.
and biological conversion. The substrate made of *F. moluccana* produced the highest biological conversion, indicating that it is the most suitable tree species that can be used for making the growing substrate for *A. polytricha* cultivation. In conclusion, the substrate made of *F. moluccana* resulted in the highest fruiting body yield. Furthermore, the period required for fruiting body production was the shortest. The high fruiting body yield is due to the great amount of mycelium in the substrate.

during cultivation. Therefore, among the 3 Indonesian commercial plantation species, *F. moluccana* wood meal is considered to be the most suitable substrate material for *A. polytricha* cultivation.

**LITERATURE CITED**
