Fungal Biology 122 (2018) 293–301

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Development of antler-type fruiting bodies of *Ganoderma lucidum* and determination of its biochemical properties

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ARTICLE INFO

Article history:
Received 17 October 2017
Received in revised form
29 January 2018
Accepted 30 January 2018
Available online 8 February 2018

Corresponding Editor: Nicholas P Money

Keywords:
Antioxidant
Bioactive compounds
G. lucidum
Growth conditions
Lingzhi
Mushroom

ABSTRACT

Following the importance of antler-type fruiting bodies of *Ganoderma lucidum*, in this study, the impact of main growth parameters such as ventilation and light on the development of antler-type fruiting bodies has been investigated together with the determination of physico-chemical properties of antler fruiting bodies. For this, the primordia bags of *G. lucidum* were kept under controlled ventilation to adjust the CO_2 produced by the mushrooms owing to its respiration under light and dark conditions. The bioactive compounds such as phenolics, flavonoids, water-soluble polysaccharides and ganoderic acid showed a two-fold increase in the antler-type fruiting bodies as compared to normal kidney-shaped fruiting bodies. It is assumed from this study that the antler type fruiting bodies are developed due to restricted ventilation which causes an increase in the level of CO_2 gas in the air as a result of respiration of mushroom. The shape and colour of antler fruiting bodies again dependent on the light provided in the growth chamber. This study also proves that with the manipulation of light and ventilation antler-type fruiting bodies of *G. lucidum* could be developed with higher quantity of bioactive compounds and with higher antioxidant potential.

1. Introduction

Ganoderma lucidum, generally known as the Lingzi mushroom, is often used in traditional Chinese medicine. *G. lucidum* has been widely used for the general promotion of health and longevity in Asian countries. The dried powder of *G. lucidum* was popular as a cancer chemotherapy agent in ancient China. It is a large, dark red kidney shaped mushroom with a glossy exterior and a woody texture. But a change in growth conditions may bring deformed mushrooms named as “Antler”. The information regarding the cultivation of antler-type fruiting bodies are least reported in the literature. Development of antler-type fruiting bodies is reported by Jo et al. (2013) where they incorporated food waste components in sawdust medium. But the details behind the antler development from the substrate have not been discussed. They used a composition of a mixture containing food waste compost (FWC), rice bran (RB), and oak sawdust (SD). The content of 15 % FWC gave the highest FB yield (27.0 ± 1.3 g/bottle), which was 44 % higher than the yield (18.6 ± 2.8 g/bottle) of their control treatments. Effects of light and ventilation on the formation of atypical fruiting structures (AFSs) and fruit body primordia (FBPs) of *G. lucidum* on nutrient agar media were investigated earlier by Seo et al. (1995). They observed that mycelial growth was inhibited by illumination as well as ventilation, which lead to the development of brown AFSs and later basidia and basidiospores were produced on it. On the other hand, FBPs were induced by illumination alone, regardless of ventilation. However, the primordia could not develop to mature fruit bodies and they observed that only vegetative growth of the fungus progressed under dark conditions.

In Japanese antler-type fruiting bodies are called as “rokkakur-eishi” (Nahata, 2013). Nutritional value of antler-type fruiting bodies of *G. lucidum* was investigated by Ulziijargal and Mau (2011)

Abbreviations: PDA, potato dextrose agar; BE, biological efficiency; TPC, total phenolic content; DPPH, 2,2-diphenylpicrylhydrazyl; FRAP, Ferric reducing ability of plasma; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; RPM, revolutions per minute; GAE, gallic acid equivalent; ROS, reactive oxygen species.

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https://doi.org/10.1016/j.funbio.2018.01.007
1878-6146/

where they reported about the proximate analysis of antler as well as normal fruiting bodies of *G. lucidum*. Antler-types had higher nutritional composition than the normal-types. Similarly, Mau et al. (2001) reported more carbohydrates and fibre content in the antler-type *G. lucidum* than the normal fruiting bodies.

Antler-type fruiting bodies have been investigated for *in vitro* studies and clinical trials. Immune potentiating effects of antler-shaped *G. lucidum* were reported by Kohguchi et al. (2004). They orally administrated antler-shaped fruiting body extract for 3 d into BALB/c mice and observed the production of interferon-gamma by spenocytes, suggesting that splenic macrophages and T cells were activated by the administration of extracts of antler fruiting body. Nonaka et al. (2008) reported the alleviation of cyclophosphamide induced immune depression and evaluated the anti-tumor and anti-metastatic effects of antler forms of *G. lucidum* in cyclophosphamide-treated mice. Their study suggests that the antler forms are beneficial in alleviating the reduction of immune response by chemotherapeutic anti-cancer drugs. Structural characterization of a heterogalactan from antler-shaped *G. lucidum* is reported by Nara and Kato (2015). The molecular weight of the heterogalactan fraction was estimated to be 1.0×10^4 by gel filtration on Sepharose CL-6B. On this context, this study has been considered to understand the growth factors and mechanism behind the development of antler-type *G. lucidum*.

2. Methodology

2.1. Project site

The experiments were conducted at the Centre of Excellence for Postharvest Biotechnology (CEPB), School of Biosciences, University of Nottingham Malaysia campus.

2.2. Culture preparation

The mycelial strain of *G. lucidum* was obtained from Ganofarm Sdn. Bhd., Tanjung Sepat, Malaysia. Fresh mycelial cultures were obtained by subculturing on fresh potato dextrose agar (PDA) plates. Incubation temperature was set at 25 ± 2 °C for 5 d for the recovery of mycelia.

2.3. Preparation of spawn

500 g of wheat grain was boiled in 1 l of water for 30 min. The grains were blown dried in a laminar flow cabinet after draining off the water which yielded 750 g of wheat grain. The wheat grains were divided into 3 portions of 250 g and packed into three polybags. Two mycelial plugs of 6 mm from the PDA plates were inoculated into each bag and kept in the dark at 25 °C for incubation. After 14 d the wheat grains colonized by mycelia were used for inoculation on the substrate.

2.4. Substrate formulation and inoculation of spawn

Sawdust (1000 g), rice bran (100 g) and calcium carbonate (10 g) were taken in a ratio of 100:10:1. The components were then well mixed followed by the addition of 65 % water. 1000 g of substrate was then packed in polypropylene bags and closed with PVC necks which was then covered with spongy plugs and wrapped with polypropylene cover to avoid entry of water inside the bag while pasteurization. The substrate bags were pasteurized at 100 °C for 8 h using a steam chamber and then left to cool at room temperature (27 °C) for 4–6 h. 5 g of *G. lucidum* spawn was inoculated into the sterilised substrate bags in a laminar air flow cabinet and placed for the formation of mycelia in a clean and dark room maintained at 28 °C.

2.5. Design of experiment

The bags after mycelial colonization were held for primordia development and were arranged into 9 sets with 5 replicates in each for the experiments. Each set of bags were placed in transparent polycarbonate boxes (112 cm length × 47 cm width × 43 cm height) with the thickness of 5 mm. First set of bags were placed in a completely closed box without any provision for ventilation and were kept under light. Next set of bags were maintained in the same way but were kept under dark condition. Other sets of bags (6 sets) were maintained at restricted ventilation based on the level of CO_2 (0.5 %, 1 % and 2 %) gas produced as a result of mushroom respiration in the boxes. Three sets were placed under light and the rest were kept under dark. One set of bags were kept as control. The control bags were well-maintained at normal growth conditions i.e. at a humidity of 85 %, a temperature of 27 °C, with proper ventilation ($CO_2 < 0.1$ %) and light (2.98 μmol m^{-2} s^{-1}). Concentration of CO_2 and CO gases produced inside the boxes as a result of mushroom respiration were checked using a gas meter (Drager X-am® 5000 Personal Monitor, Germany). The available O_2 present in the boxes was checked with an O_2 meter (Lutron, DO-5510HA, Taiwan). The boxes with mushroom bags were exposed to light using electric fluorescent lamps throughout the experiments. The light provided to the mushrooms was checked with a light meter (Skye Instruments, 660/730 sensor, Wales) by measuring the red light (660 nm)/far red light (730 nm) ratio at 0–200 m range. Inside the box the humidity was maintained by keeping a beaker filled with water (100 ml). The humidity inside the box was checked using a humidity meter (Fischer Scientific 433 MHz cable free traceable meter, China). The growth of antler-type and normal kidney-shaped fruiting bodies were checked every day. The antler-types showed indefinite growth of their fruiting bodies irrespective of the normal fruiting bodies of *G. lucidum* which have precise harvest time and make it difficult to calculate the harvesting time of antler-type fruiting bodies. Therefore, the fruiting bodies of antler were harvested at the same time (120 d) at which the normal fruiting bodies are harvested. The fruiting bodies soon after the harvest were taken for calculating the biological efficiency and chemical properties.

2.6. Biological efficiency

The biological efficiency was calculated based on the following Equation (A.1) as described by Chang et al. (1981),

$$B.E(\%) = \text{Fresh weight of mushroom/dry weight of substrate*100} \quad \text{(A.1)}$$

2.7. Chemical properties

2.7.1. Sample preparation for extraction

Immediately after harvesting the fruiting bodies of *G. lucidum* were preserved by drying in a Freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbHChrist, Germany) for 24 h at a freezing temperature of −50 °C. Mushroom powder with the particle size of 1 mm was obtained by grinding in a commercial blender (Waring Commercial, USA, Model HGB2WTS3) which was then stored in air-tight sealed plastic bags and kept in a desiccator at room temperature.